

May 2025 Office of Chemical Safety and Pollution Prevention

Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Diethylhexyl Phthalate (DEHP) (1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester)

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 117-81-7



May 2025

This supplemental file contains information regarding the data quality evaluation conducted for references that (1) met PECO screening criteria, (2) were published prior to 2014 which was the preferred literature cutoff date by EPA for data reported in previous assessments, and (3) reported human equivalent dose (HED) derived from points of departure (POD) that contained lowest-observable-effect levels (LOEL) greater than an order of magnitude of the lowest HED lowest-observable-adverse-effect level (LOAEL) identified across existing assessments. For a detailed description on these three criteria, see the *Draft Risk Evaluation for Diethylhexyl Phthalate (DEHP) – Systematic Review Protocol*. EPA conducted data quality evaluation based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration into the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. For the data quality evaluation, EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as '2021 Draft Systematic Review Protocol are described in the *Draft Risk Evaluation for Diethylhexyl Phthalate (DEHP) – Systematic Review Protocol*.

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Reference

Short-term (>1-30 days)		
673552	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.	7
673553	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.	9
1325511	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.	25
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679540	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.	38
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2000828	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490.	100
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673292	Lee, B. M., Koo, H. J. (2007). Hershberger assay for antiandrogenic effects of phthalates. Journal of Toxicology and Environmental Health, Part A: Current Issues 70(15-16):1365-1370.	124
674395	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.	128

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11784618	Santacruz-Márquez, R., Safar, A. M., Laws, M. J., Meling, D. D., Liu, Z., Kumar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The effects of short-term and long-term phthalate exposures on ovarian follicle growth dynamics and hormone levels in female mice ⁺ , Biology of Reproduction 110(1):198-210.	146
697420	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.	149
Subchronic (>30-91 days)		
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7978408	Gu, Y., Gao, M., Zhang, W., Yan, L., Shao, F., Zhou, J. (2021). Exposure to phthalates DEHP and DINP May lead to oxidative damage and lipidomic disruptions in mouse kidney. Chemosphere 271:129740.	163
2000828	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490.	165
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674395	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.	178
Chronic (>91 days)		
673552	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.	196
679540	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.	200
11784622	Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl phthalate, and a mixture of phthalates alters estrous cyclicity and/or impairs gestational index and birth rate in mice. Toxicological Sciences 193(1):48-61.	212
11784618	Santacruz-Márquez, R., Safar, A. M., Laws, M. J., Meling, D. D., Liu, Z., Kumar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The effects of short-term and long-term phthalate exposures on ovarian follicle growth dynamics and hormone levels in female mice [†] . Biology of Reproduction 110(1):198-210.	218
Reproductive/Developmental		
673553	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.	221
673565	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-97.	229
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698207	Culty, M., Thuillier, R., Li, W., Wang, Y., Martinez-Arguelles, D., Benjamin, C., Triantafilou, K., Zirkin, B., Papadopoulos, V. (2008). In utero exposure to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat. Biology of Reproduction 78(6):1018-1028.	249			
9419406	Gray, L. E., Jr, Lambright, C. S., Conley, J. M., Evans, N., Furr, J. R., Hannas, B. R., Wilson, V. S., Sampson, H., Foster, D., P.M. (2021). Genomic and Hormonal Biomarkers of Phthalate-Induced Male Rat Reproductive Developmental Toxicity Part II: A Targeted RT-qPCR Array Approach That Defines a Unique Adverse Outcome Pathway. Toxicological Sciences 182(2):195-214.	251			
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674193	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). Differential prenatal toxicity of branched phthalate esters in rats. Food and Chemical Toxicology 35(5):501-512.	271			
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698185	Lin, H., Ge, R., Chen, G., Hu, G., Dong, L., Lian, Q., Hardy, D., Sottas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in fetal Leydig cell aggregation after exposure to phthalate in utero. Proceedings of the National Academy of Sciences of the United States of America 105(20):7218-7222.	305			
697737	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888.	310			
680063	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.	315			
676281	Martino-Andrade, A. J., Morais, R. N., Botelho, G. G., Muller, G., Grande, S. W., Carpentieri, G. B., Leao, G. M., Dalsenter, P. R. (2008). Coadministration of active phthalates results in disruption of foetal testicular function in rats. International Journal of Andrology 32(6):704-12.	337			
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2519077	Rajesh, P., Balasubramanian, K. (2014). Phthalate exposure in utero causes epigenetic changes and impairs insulin signalling. Journal of Endocrinology 223(1):47-66.	342			
2000935	Saillenfait, A. M., Sabaté, J. P., Robert, A., Rouiller-Fabre, V., Roudot, A. C., Moison, D., Denis, F. (2013). Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. Journal of Applied Toxicology 33(9):1027-1035.	345			
732820	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506.	348			
3108900	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report.	355			

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697710	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411.	406
2519060	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221.	416

Study Citation:	Akingbemi,	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple				
Health Outcome(s) and Reported Health Effect(s):	endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780. Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatage gene expression in Leydig cells					
Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)				
Species:	Rat-Long-E	vans - [rat]-Male				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	673552					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	Low	The study does not report how animals were allocated to study groups. No other meth- ods to control for modifying factors across groups were noted.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).		
Domain 2: Confounding	Voriabla Ca	ntrol				
Domain 5. Contounding	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Food and water dispensing containers were not described.		
Domain 4: Selective Re	porting and At	trition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for all prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.		
Domain 5: Exposure M	ethods Sensitiv	vity				
		Contin	ued on next pa			

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673552 Table: 1 of 1

		conti	inued from previ	ous page			
Study Citation:	Akingbemi, endocrine d	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.					
Health Outcome(s)	Reproductiv	Reproductive/Developmental-Testicular weight serum estradiol testosterone and luteinizing hormone levels: ex vivo production of testosterone and					
and Reported	estradiol fro	estradiol from isolated Levdig cells (basal and after LH stimulation): Levdig cell proliferation (assessed by 1) mRNA expression of cell division cycle					
Health Effect(s):	markers (PC	markers (PCNA cyclin D3 and G1 and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the					
ficultin Effect(5)	number of I	number of Levdig cells in testis by stereology): aromatase gene expression in Levdig cells					
Duration and	Oral-Gavag	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)					
Exposure Route:	orar ourag		uuj (8)				
Snecies:	Rat-Long-E	vans - [rat]-Male					
Chemical	Diethylhexy	/l Phthalate- Parent compound					
HFRO ID.	673552	T Thinking T arent compound					
	073332						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and	Low	The source and purity of the test substance were not reported. Gavage volume was not			
		characterization		reported. No information is provided on preparation or storage of the test substance.			
				It is unclear now far in advance solutions were made. Only target concentrations are			
	Metric 7	Exposure timing frequency and	High	The timing and duration of exposure were appropriate for the outcomes of interest			
	Metric 7.	duration	Ingn	The timing and duration of exposure were appropriate for the outcomes of interest.			
		duration					
Domain 6: Outcome Me	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group			
			8	sizes were large enough and sufficient for statistical analysis. The doses were chosen			
				based on reported findings by this study group. Assessment of endpoints were appropri-			
				ate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	Medium	Results for most endpoints were described in the text and data were presented in tables			
				as means \pm standard error. Statistical tests were reported and appropriate. Histopatho-			
				logical data were reported as negative in the text.			
Additional Comments:	Study inclue	ded 3 different durations of exposure during	post weaning up	to adulthood. each "experiment" included different outcomes and were potentially			
	performed of	on different cohorts of animals (though thi	s is unclear and	all groups were >10). not all outcomes were evaluated for each duration. This			
	evaluation corresponds with the PND21-48 duration						

Medium

Study Citation:	Akingbemi, steroidogeni	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.					
Health Outcome(s)	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal						
Health Effect(s):	vesicles weight						
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-14-da	y(s)-Oral-Gava	ge-Duration: Short-term (>1-30 days)-7-14-day(s)			
Exposure Route:							
Species:	Rat-Long-E	vans - [rat]-Male					
Chemical: HFRO ID:	Diethylhexy 673553	I Phthalate- Parent compound					
Domain	013333	Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	d Performance	Allocation	High				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu- tion between groups".			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.			
Domain 4: Selective Re	porting and At	trition					
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.			

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Diethylhexyl Phthalate

		cont	inued from previ	ous page	
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight				
Exposure Route:	Ofal-Gavag	e-Duration. Short-term (>1-50 days)-7-14-	-day(s)-Orai-Gava	ge-Duration. Short-term ($>1-50$ days)- $(-14-day(s))$	
Species:	Rat-Long-E	Evans - [rat]-Male			
Chemical:	Diethylhexy	yl Phthalate- Parent compound			
HERO ID:	673553				
Domain		Metric	Rating	Comments	
Domain 5: Exposure Me	ethods Sensiti	vity			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.	
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproduc- tive effects. Exposure was consistent across study groups. Groups were treated concur- rently.	
Domain 6: Outaoma Ma	acurac and De	aulta Display			
Domain 6: Outcome Me	easures and Ke	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data). Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.	
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means \pm SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.	
Additional Comments:	None				
Overall Qualit	ty Deter	mination	Medium		

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Study Citation: Health Outcome(s) and Reported Health Effect(s):	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight					
Duration and Exposure Poute:	Oral-Gavage	-Duration: Short-term (>1-30 days)-7-14-da	iy(s)-Oral-Gava	ge-Duration: Short-term (>1-30 days)-7-14-day(s)		
Species: Chemical: HERO ID:	Rat-Long-Ev Diethylhexyl 673553	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 673553				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu- tion between groups".		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)		
Domain 3: Confounding	y / Variable Cor	atrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.		
Domain A: Selective Deporting and Attrition						
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.		
Domain 5: Exposure Me	ethods Sensitiv	ity				
		Contin	ued on next pa			

Diethylhexyl Phthalate

		cont	inued from previ	ous page				
Study Citation:	Akingbemi, steroidogeni	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylbexyl)phthalate. Biology of Reproduction 65(4):1252-1259.						
Health Outcome(s)	Reproductiv	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal						
and Reported	vesicles wei	vesicles weight Oral Gavage Duration: Short term (>1.30 days) 7.14 day(s) Oral Gavage Duration: Short term (>1.30 days) 7.14 day(s)						
Health Effect(s):	Oral Cavage							
Exposure Route:	Ofal-Oavage	Oral-Oavage-Duration. Short-term (>1-50 days)-7-14-day(s)-Oral-Oavage-Duration. Short-term (>1-50 days)-7-14-day(s)						
Species:	Rat-Long-E	Rat-Long-Evans - [rat]-Male						
Chemical:	Diethylhexy	l Phthalate- Parent compound						
HERO ID:	673553							
Domain		Metric	Rating	Comments				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.				
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproduc- tive effects. Exposure was consistent across study groups. Groups were treated concur- rently.				
Domain 6: Outcome M	easures and Re	sults Display						
Domain 0. Outcome init	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data). Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.				
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means \pm SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.				
Additional Comments:	None							

Overall Quality Determination

Medium

Study Citation:	Akingbemi,	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell					
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight						
Duration and	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)					
Exposure Route:	Pot Long F	vans [rat] Mala					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	673553						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported; qualitative results were reported for some, but not all outcomes.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu- tion between groups".			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, organ weights)			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.			
Domain 4: Selective Re	porting and At	trition					
	Metric 5:	Selective Reporting and Attrition	Medium	Data were qualitatively reported for some reproductive outcomes in the text. Organ weight results were not reported. The text specified the number of animals included in each group, it is not clear whether the sample sizes were equal to the number of animals per group.			
Domain 5: Exposure M	ethods Sensitiv	ity					
Continued on next page							

Diethylhexyl Phthalate

		conti	inued from previ	ous page	
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species:	Akingbemi, steroidogeni Reproductiv vesicles wei Oral-Gavage Rat-Long-E	 Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s) 			
Chemical: HERO ID:	Diethylhexyl Phthalate- Parent compound 673553				
Domain		Metric	Rating	Comments	
	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported. Exposure was during PND 62-89 which is the period relevant for male reproductive	
		duration	ingn	tract development. Exposure was consistent across study groups. Groups were treated concurrently.	
Domain 6: Outcome M	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focuses on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not specified. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.	
	Metric 9:	Results presentation	Low	Data were not shown for any endpoint. Results were only reported in the text stating that no effects were reportedly observed; however, no results for organ weights were described in the text. Statistical analysis methods were reported.	
Additional Comments:	None				

Overall Quality Determination

Medium

Study Citation:	Akingbemi, I	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylbexyl)phthalate. Biology of Reproduction 65(4):1252-1259.					
Health Outcome(s) and Reported Health Effect(s):	Reproductive vesicles weig	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight					
Duration and	Oral-Gavage	-Duration: Short-term (>1-30 days)-7-28-da	ay(s)				
Exposure Route:							
Species: Chemical:	Rat-Long-Ev Diethylhexyl	ans - [rat]-Male Phthalate- Parent compound					
HERO ID:	673553	The second sec					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quanty	Medium	The test substance was identified by name di(2-ethylnexyl)phthalate (DEHP). A CASKN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	l Performance						
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu- tion between groups".			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)			
Domain 3: Confounding	g / Variable Con	trol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.			
Domain 4: Selective Re	porting and Att	rition					
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for all outcomes in text or table. The number of animals per group was reported and consistent and all animals were used to assess each endpoint. There was no indication of selective reporting/attrition.			
Domain 5: Exposure Mo	ethods Sensitivi	ty					
	Continued on next page						

Diethylhexyl Phthalate

		conti	inued from previ	ous page			
Study Citation: Health Outcome(s) and Reported Health Effect(a):	Akingbemi, steroidogen Reproductiv vesicles wei	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight					
Duration and Exposure Route:	Oral-Gavag	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)					
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673553	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 673553					
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproduc- tive effects. Exposure was consistent across study groups. Groups were treated concur- rently.			
Domain 6: Outcome Me	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified al-though previous studies using DEHP and a developmental study using another phthalate were consulted.			
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means \pm SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.			
Additional Comments:	None						
Overall Qualit	ty Deteri	nination	Medium				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.					
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route:	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)					
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673553	vans - [rat]-Male l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu-		
	Metric 3:	Observational Bias / Blinding Changes	Medium	tion between groups". Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)		
Domain 3: Confounding	a / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. These missing details are not ex- pected to have a significant impact on the selected endpoints.		
Domain 4: Selective Re	porting and At Metric 5:	trition Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.		
Domain 5: Exposure M	ethods Sensitiv	ity Contin	ued on next pa	Ige		

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 673553 Table: 5 of 8

		conti	inued from previ	ous page			
Study Citation: Health Outcome(s) and Reported	Akingbemi, steroidogen Nutritional/	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats					
Health Effect(s): Duration and Exposure Route:	Oral-Gavag	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)					
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673553	vans - [rat]-Male /l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproduc- tive effects. Exposure was consistent across study groups. Groups were treated concur- rently.			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data), but were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.			
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means \pm SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.			
Additional Comments:	None						
Overall Quali	ty Deter	mination	Medium				

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.					
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route:	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)					
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673553	vans - [rat]-Male l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu-		
	Metric 3:	Observational Bias / Blinding Changes	Medium	tion between groups". Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)		
Domain 3: Confounding	a / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. These missing details are not ex- pected to have a significant impact on the selected endpoints.		
Domain 4: Selective Re	porting and At Metric 5:	trition Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.		
Domain 5: Exposure M	ethods Sensitiv	ity Contin	ued on next pa	Ige		

May 2025

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 673553 Table: 6 of 8

		conti	nued from previ	ious page			
Study Citation: Health Outcome(s) and Reported	Akingbemi, steroidogen Nutritional/	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats					
Health Effect(s): Duration and Exposure Route:	Oral-Gavag	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)					
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673553	vans - [rat]-Male /l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproduc- tive effects. Exposure was consistent across study groups. Groups were treated concur- rently.			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data), but were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.			
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means \pm SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.			
Additional Comments:	None						
Overall Quali	ty Deter	mination	Medium				

Study Citation:	Akingbeni, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell				
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route:	steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)				
Species:	Rat-Long-Ev	vans - [rat]-Male			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	673553				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported; qualitative results were reported for some, but not all outcomes.	
Domain 2: Selection an	d Performance				
Domain 2. Selection an	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu- tion between groups".	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, organ weights)	
Danain 2. Canfanadia					
Domani 5: Contounding	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food contain- ers, and water dispensing containers were not described. These missing details are not expected to have a significant impact on the interpretation of the results for these end- points.	
	,• • • • • •	, ·.·			
Domain 4: Selective Re	Metric 5:	Selective Reporting and Attrition	Medium	Data were qualitatively reported for some reproductive outcomes in the text. Organ weight results were not reported. The text specified the number of animals included in each group, it is not clear whether the sample sizes were equal to the number of animals per group.	
Domain 5: Exposure M	ethods Sensitiv	ity			
		Contin	ued on next pa	age	

Diethylhexyl Phthalate

		cont	tinued from previo	ous page			
Study Citation: Health Outcome(s)	Akingbemi, steroidogeni Nutritional/N	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats.					
and Reported Health Effect(s):		Nutritional/Metabolic-body weight and lood consumption in dams and young adult rats					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-28	-day(s)				
Species: Chemical: HERO ID:	Rat-Long-Ev Diethylhexy 673553	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 673553					
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during PND 62-89 which is the period relevant for male reproductive tract development. Exposure was consistent across study groups. Groups were treated concurrently.			
Domain 6: Outcome Me	easures and Rea	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focuses on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not specified. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.			
	Metric 9:	Results presentation	Low	Data were not shown for any endpoint. Results were only reported in the text stating that no effects were reportedly observed; however, no results for organ weights were described in the text. Statistical analysis methods were reported.			
Additional Comments:	None						

Overall Quality Determination

Medium

Study Citation:	Akingbemi,	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell					
Health Outcome(s) and Reported Health Effect(s):	Nutritional/	steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats					
Duration and Exposure Poute:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-28-da	ay(s)				
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673553	vans - [rat]-Male /l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to "ensure equal weight distribu- tion between groups".			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)			
Domain 3: Confounding	o / Variable Co	ontrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. This is not expected to have a sig- nificant impact on the endpoint of interest (e.g., body weight)			
Domain 4: Selective Re	Domain 4: Selective Reporting and Attrition						
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for all outcomes in text or table. The number of animals per group was reported and consistent and all animals were used to assess each endpoint. There was no indication of selective reporting/attrition.			
Domain 5: Exposure M	ethods Sensitiv	vity					
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Diethylhexyl Phthalate

continued from previous page						
Study Citation: Health Outcome(s) and Reported Health Effect(c):	Akingbemi, F steroidogenic Nutritional/M	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats				
Duration and	Oral-Gavage-	Duration: Short-term (>1-30 days)-7-28-day(s)			
Exposure Route: Species: Chemical: HERO ID:	Rat-Long-Eva Diethylhexyl 673553	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 673553				
Domain		Metric	Rating	Comments		
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.		
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is appropriate for the endpoints of inter- est (e.g., body weights)		
Domain 6: Outcome Me	asures and Resi	ults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. The methods did not specify the timing of body weight and feed intake measurements, but the results noted body weights were measured at the beginning and end of the exposure. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.		
	Metric 9:	Results presentation	Medium	Results were reported in tables for body weights (shown as means \pm SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.		

Additional Comments: None

Overall Quality Determination

Medium

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID: Domain	 BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter. Renal/Kidney-Kidney weight and histology-Reproductive/Developmental-Testis weight and histology-Hepatic/Liver-Liver weight and histology. Serum triglyceride and total cholesterol. Biochemical analysis of liver (cyanide-insensitive palmitoyl-CoA oxidation and protein concentration; microsomal fraction rate of lauric acid 11-hydroxylase and 12-hydroxylase activity) and ultrastructure of liver assessing peroxisome proliferation (TEM)-Mortality-Mortality Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s) Rat-Fischer 344 - [rat]-Both Diethylhexyl Phthalate- Parent compound 1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547 					
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was identified as along with the source. Purity was not reported. Test animal species, strain, sex, age, initial body weight and source were reported. Husbandry conditions (temperature, hu- midity, and light cycle) were reported. Animals were individually housed. Food and water were available ad libitum. The dose levels, frequency, duration, and route of expo- sure were reported. Food intake and body weights were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not ex- pected to significantly impact the study evaluation.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups by use of random number tables. Group weights were checked, and further randomization was made if a significantly unequal distribution was identified.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., body weight, organ weights, clinical chemistry) or consisted of initial histopathology review, and no secondary histopathology review was conducted.		
Domain 3: Confounding	g / Variable Cor	ntrol				
	Metric 4:	Confounding / Variable Control	Low	A negative and positive control group were included, and responses were appropriate. Water was delivered in glass bottles with stainless-steel drinking nozzles eliminating potential confounding from phthalates leaching into water from plastic water bottles. Food and water were analyzed for contamination and authors conclude "contaminates present in food and water are unlikely to adversely affect the outcome of the study". There was marked differences in food intake between the groups. Food intake was sig- nificantly reduced (>20% difference from control at some points), this could have led to malnourishment in these animals and potentially confounding the results. The data suggest palatability issues with diet since reduction in food intake occurred during the first week.		

Domain 4: Selective Reporting and Attrition

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Diethylhexyl Phthalate

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HERO ID: 1325511 Table: 1 of 3

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter. Renal/Kidney-Kidney weight and histology-Reproductive/Developmental-Testis weight and histology-Hepatic/Liver-Liver weight and histology. Serum triglyceride and total cholesterol. Biochemical analysis of liver (cyanide-insensitive palmitoyl-CoA oxidation and protein concentration; microsomal fraction rate of lauric acid 11-hydroxylase and 12-hydroxylase activity) and ultrastructure of liver assessing peroxisome proliferation (TEM)-Mortality-Mortality Oral-Diet-Duration; Short-term (>1-30 days)-7-24-21-day(s)				
Exposure Route:					
Species:	Rat-Fischer	344 - [rat]-Both			
Chemical:	Diethylhexy	l Phthalate- Parent compound bled HEPO ID(a): 1325511 674033 1325	463 1325547		
Domain	1525511 LII	Metric	Rating	Comments	
Domain	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in results. There is no indication that treated animals were excluded from analysis.	
Domain 5: Exposure Me	ethods Sensitiv Metric 6:	vity Chemical administration and characterization	Low	Purity of test substance was not reported. Diets were analyzed for concentration of test substance (not reported) but were deemed acceptable if concentration was within 5% of target concentration and coefficient of variation between samples was <10%. Preparation of diet with test substance was not fully reported. Stability tests were performed by authors or study sponsor which determined how often diets would be prepared (approximately one week in advance or shorter). Study authors calculated doses based on food intake and body weights.	
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency and duration were acceptable for the endpoints of inter- est. Young rats were chosen since they are known to be susceptible to the induction of peroxisomes, which was the primary aim of the study.	
Domain 6: Outcome Me	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was pro- vided. The outcome methodology addressed the intended outcomes of interest and as- sessed consistently across the study groups. Organ weighs and histology (liver, kidney, testis) and serum triglycerides and total cholesterol. The number of animals/group was appropriate (n=5/sex/group).	
	Metric 9:	Results presentation	High	Data were reported with means and standard error or incidence of histological findings. Statistical analysis was reported and appropriate. No deaths were reported, all animals were accounted for in the results.	
Additional Comments:	None				
Overall Qualit	ty Deterr	nination	Medium		

Study Citation: Health Outcome(s) and Reported	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter. Other (please specify below) (Clinical signs)-Clinical signs of toxicity					
Duration and Exposure Route:	Oral-Diet-Du	rration: Short-term (>1-30 days)-7-24-21-day((s)			
Species: Chemical: HERO ID:	Rat-Fischer 3 Diethylhexyl 1325511 Lin	344 - [rat]-Both Phthalate- Parent compound ked HERO ID(s): 1325511, 674933, 1325463,	1325547			
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was identified as along with the source. Purity was not reported. Test animal species, strain, sex, age, initial body weight and source were reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were individually housed. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Food intake and body weights were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups by use of random number tables. Group weights were checked, and further randomization was made if a significantly unequal distribution was identified.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for evaluation of clinical signs.		
Domain 3: Confounding	v / Variable Cor	atral				
	Metric 4:	Confounding / Variable Control	Low	A negative and positive control group were included, and responses were appropriate. Water was delivered in glass bottles with stainless-steel drinking nozzles eliminating potential confounding from phthalates leaching into water from plastic water bottles. Food and water were analyzed for contamination and authors conclude "contaminates present in food and water are unlikely to adversely affect the outcome of the study". There was marked differences in food intake between the groups. Food intake was significantly reduced (>20% difference from control), this could have led to malnourishment in these animals and potentially confounding the results.		
Domain 4: Selective Re	porting and Att	rition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in results. There is no indication that treated animals were excluded from analysis.		
Domain 5: Exposure Mo	ethods Sensitivi	ity				
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Diethylhexyl Phthalate

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HERO ID: 1325511 Table: 2 of 3

		Ci	ontinued from previous	page			
Study Citation: Health Outcome(s) and Reported	BIBRA, (19 Other (pleas	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter. Other (please specify below) (Clinical signs)-Clinical signs of toxicity					
Health Effect(s): Duration and Exposure Route:	Oral-Diet-D	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)					
Species: Chemical	Diethylhexy	744 - [Tat]-Dolli 1 Phthalate- Parent compound					
HERO ID:	1325511 Li	nked HERO ID(s): 1325511, 674933, 132546	53, 1325547				
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	Purity of test substance was not reported. Diets were analyzed for concentration of test substance (not reported) but were deemed acceptable if concentration was within 5% of target concentration. and coefficient of variation between samples was <10%. Preparation of diet with test substance was not fully reported. Stability tests were performed by authors or study sponsor which determined how often diets would be prepared (approximately one week in advance or shorter). Study authors calculated doses based on food intake and body weights.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency and duration were acceptable for the endpoints of inter- est. Young rats were chosen since they are known to be susceptible to the induction of peroxisomes, which was the primary aim of the study.			
Domain 6: Outcome M	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was pro- vided. The outcome methodology addressed the intended outcomes of interest and assessed consistently across the study groups. The number of animals/group was ap- propriate (n=5/sex/group).			
	Metric 9:	Results presentation	Uninformative	No information was provided on clinical signs.			
Additional Comments:	None						
Overall Quali	ty Deteri	nination	Uninformative	9			

Study Citation: Health Outcome(s) and Reported	BIBRA, (198 Nutritional/N	36). Rat liver and lipid effects of representative Metabolic-Body weight and food intake	e phthalate esters with H	EPA acknowlegement letter.
Health Effect(s): Duration and Exposure Route:	Oral-Diet-Du	uration: Short-term (>1-30 days)-7-24-21-day	(s)	
Species: Chemical: HERO ID:	Rat-Fischer 3 Diethylhexyl 1325511 Lin	344 - [rat]-Both Phthalate- Parent compound ked HERO ID(s): 1325511, 674933, 1325463	, 1325547	
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	Juality			
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was identified as along with the source. Purity was not reported. Test animal species, strain, sex, age, initial body weight and source were reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were individually housed. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Food intake and body weights were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection an	d Performance			
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups by use of random number tables. Group weights were checked, and further randomization was made if a significantly unequal distribution was identified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., body weight, organ weights, clinical chemistry) or consisted of initial histopathology review, and no secondary histopathology review was conducted.
Domain 3: Confoundin	a / Variable Cor	atrol		
	Metric 4:	Confounding / Variable Control	Uninformative	A negative and positive control group were included, and responses were appropriate. Water was delivered in glass bottles with stainless-steel drinking nozzles eliminating potential confounding from phthalates leaching into water from plastic water bottles. Food and water were analyzed for contamination and authors conclude "contaminates present in food and water are unlikely to adversely affect the outcome of the study". There was marked differences in food intake between the groups. Food intake was significantly reduced (>20% difference from control at some points), this could have led to malnourishment in these animals and potentially confounding the results. The data suggest palatability issues with diet since reduction in food intake occurred during the first week. Based on the significant decrease in food intake, this would substantially impact body weight outcomes. Therefore, this study was deemed uninformative for body weight outcomes.
Domain 4: Selective Re	eporting and Att Metric 5:	rition Selective Reporting and Attrition	High	All animals were accounted for in results. There is no indication that treated animals were excluded from analysis.
		Сог	ntinued on next page .	

Diethylhexyl Phthalate

		сог	ntinued from previou	s page				
Study Citation: Health Outcome(s) and Reported	BIBRA, (19 Nutritional/I	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter. Nutritional/Metabolic-Body weight and food intake						
Health Effect(s): Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)							
Species: Chemical: HERO ID:	Rat-Fischer Diethylhexy 1325511 Lii	Rat-Fischer 344 - [rat]-Both Diethylhexyl Phthalate- Parent compound 1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547						
Domain		Metric	Rating	Comments				
Domain 5: Exposure Me	ethods Sensitiv Metric 6: Metric 7:	vity Chemical administration and characterization Exposure timing, frequency, and	Low High	Purity of test substance was not reported. Diets were analyzed for concentration of test substance (not reported) but were deemed acceptable if concentration was within 5% of target concentration and coefficient of variation between samples was <10%. Preparation of diet with test substance was not fully reported. Stability tests were performed by authors or study sponsor which determined how often diets would be prepared (approximately one week in advance or shorter). Study authors calculated doses based on food intake and body weights.				
		duration	8	est. Young rats were chosen since they are known to be susceptible to the induction of peroxisomes, which was the primary aim of the study.				
Domain 6: Outcome Me	easures and Re	sults Display						
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was pro- vided. The outcome methodology addressed the intended outcomes of interest and as- sessed consistently across the study groups. Organ weighs and histology (liver, kidney, testis) and serum triglycerides and total cholesterol. The number of animals/group was appropriate (n=5/sex/group).				
	Metric 9:	Results presentation	High	Data were reported with means and standard error. Statistical analysis was reported and appropriate. No deaths were reported, all animals were accounted for in the results.				
Additional Comments:	None							

Overall Quality Determination

Uninformative

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Chiang, C., Lewis, L. R., Borkowski, G., Flaws, J. A. (2020). Exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood disrupts hormones and ovarian folliculogenesis throughout the prime reproductive life of the mouse. Toxicology and Applied Pharmacology 393:114952. Reproductive/Developmental-Following 10 days of exposure at various post-dosing time points (e.g., immediately post-dosing, 3-, 6-, and 9-months post-dosing depending on the experiments) histological analysis of the follicular development in ovarian tissue samples and the sex hormone present in sera (e.g., testosterone, progesterone, estradiol, FSH, and Inhibin B) from adult female mice were analyzed. Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s) Mouse-CD-1 - [mouse]-Female Diethylhexyl Phthalate- Parent compound 7978479 				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test animals used were CD-1 female mice purchased from Charles Rivers (Wilmington MA). These mice were maintained in ideal conditions for temperature (21.1 \pm 2.2 °C), humidity (50 \pm 20 %), access to food (ad libitum), number of animals per cage (3 animals/cage), and day/night cycles (12h/12hr). Mice were housed 3 to a cage, with all doses be in the same cage to avoid cross-contamination. All procedures were approved by the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee (Protocol No.: 17079). The test chemical (DEHP) was purchased from Sigma Aldrich (St. Louis, MO), however the CASN was not provided. The study doses animals orally via insertion of a pipette tip into the mouth, utilizes a control (corn oil vehicle), and includes a large range of doses: DEHP (20 $\mu g/kg/day$, 200 $\mu g/kg/day$, 20 mg/kg/day). Dosing occurred at PND 39-40 for 10 days followed by various post-dosing assessments for reproduction/developmental endpoints. The timepoints for endpoint collection was either immediately, 3-, 6-, or 9-months post-exposure. To analyze the ovarian follicle development, the authors utilized hematoxylin and eosin stains of tissue samples, categorizing the follicles into stages (primordial, primary, preantral, or antral) and allowing for blinded counting. Sex hormones in blood were analyzed using either commercially available enzyme-linked immunosorbent assays (ELISAs) or sent to the University of Virginia Center for Research in ReproductionLigand Assay and Analysis Core for radioimmunoassay and ELISA.	
Domain 2: Selection and	l Performance Metric 2:	Allocation	Low	There is no explicit language indicating use of randomization for allocating animals to	
	Metric 3:	Observational Bias / Blinding Changes	High	groups to reduce bias in this study. The use of blinding was used in histological experiments. To blind counters to treatment groups and avoid bias, ovaries were given a unique histological ID with no relation to treatment group. Other metrics did not state similar blinding, however, the experimen- tal/technical controls for sex hormone levels in sera are considered sufficient for proper analysis.	
Domain 3: Confounding / Variable Control					
Continued on next page					

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HERO ID: 7978479 Table: 1 of 1

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	Chiang, C., hormones an Reproductiv dosing depe (e.g., testost Oral-Gavage Mouse-CD- Diethylhexy	Lewis, L. R., Borkowski, G., Flaws, J. A. nd ovarian folliculogenesis throughout the ve/Developmental-Following 10 days of ex ending on the experiments) histological ar erone, progesterone, estradiol, FSH, and I e-Duration: Short-term (>1-30 days)-7-10 1 - [mouse]-Female vl Phthalate- Parent compound	. (2020). Exposure prime reproductiv coosure at various p nalysis of the follio nhibin B) from adu)-day(s)	e to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood disrupts e life of the mouse. Toxicology and Applied Pharmacology 393:114952. post-dosing time points (e.g., immediately post-dosing, 3-, 6-, and 9-months post- cular development in ovarian tissue samples and the sex hormone present in sera alt female mice were analyzed.	
HERO ID:	/9/84/9				
Domain	Metric 4:	Metric Confounding / Variable Control	Rating Medium	Comments The study has minor confounds that may have minimally affected the results. For ex- ample, there is no indication of using randomization when assigning mice to their ex- perimental group. Also, the chemical being used is not listed with all the relevant infor- mation regarding it's purity and measures were not taken to ensure the dose given to the mice was delivered sufficiently. However, measures were taken to reduce variability and bias such as collecting all tissue and samples during the diestrus phase.	
Domain 4: Selective Re	eporting and At Metric 5:	ttrition Selective Reporting and Attrition	Medium	The authors do explicitly state that 5 animal throughout all the groups were removed from the study because they were either found dead or were euthanized due to illness. However, there is no clear indication which groups these come from. The sample sizes per study is listed as a range and not for individual groups.	
Domain 5: Exposure M	lethods Sensitiv	vity			
	Metric 6:	Chemical administration and characterization	Medium	The chemical of interest was purchased from Sigma Aldrich (St. Louis, MO), however, neither the CASN number nor the catalog number from Sigma was indicated leaving room for speculation on the chemicals purity and composition. In a reference from the same author (HERO: 3070927) the purity of DEHP was stated to be 99%. There was no independent verification of the test substance purity, nor were there measures taken to ensure that each animal was getting their full dose. The exposure volume was determined based on body weight taken that day, indicating variable dosing volumes were possible.	
	Metric 7:	Exposure timing, frequency, and duration	High	The study doses animals orally via insertion of a pipette tip into the mouth, utilizes a control (corn oil vehicle), and includes a large range of doses: DEHP (20 $\mu g/kg/day$, 200 $\mu g/kg/day$, 20 mg/kg/day, and 200 mg/kg/day) and DINP 20 $\mu g/kg/day$, 100 $\mu g/kg/day$, 20 mg/kg/day, and 200 mg/kg/day). Dosing occurred at PND 39-40 for 10 days followed by various post-dosing assessments for ovarian follicle and sex hormone endpoints.	
Domain 6: Outcome M	Domain 6: Outcome Measures and Results Display				

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HERO ID: 7978479 Table: 1 of 1

received by the mice. The studies sample size throughout the reference varies per group, with some groups having 1-3 samples. The authors are not transparent in the sample sizes per experimental result per group, which can impact interpretation of statistical analysis. All mentions are listed as a range of values. Histological analysis of follicle development was conducted on ovarian tissues from exposed female mice with appropriate blinding to reduce bias. The criteria for designating follicle stages was listed and given proper citation. In most of these experiments, the sample size for some of the groups had a minimum of 4. The measurement of sex hormones in sera of female mice was conducted using commercially available enzyme-linked immunosorbent assays (ELISAs) or radioimmunoassays conducted by the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core. Appropriate controls and calibrations were discussed. The sample size per post-exposure time point (e.g., immediate, 3-, 6-, and 9-month post-exposure) varied between assays. For the immediate group, some measures had as low as 1 sample which were indicated by the lack of error bar. Other groups such as the 3-month and 6-month have samples sizes of as low as 3-4.

The data within the study were presented in an accurate and somewhat transparent manner. There is no clear indication what the sample size is per groups, since the authors only present the sample sizes as a range and not for each dose. Graphs depict variance as standard error bars, however, the actual SE values are not listed in the figure nor within the results section. Statistical analysis is appropriate for normal (ANOVA and a 2-sided Dunnett's pot hoc test) and non-parametric (Kruskal-Wallis test and a Mann-Whitney U

Diethylhexyl Phthalate	
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		cont	inued from previo	us page		
Study Citation:	Chiang, C., I	Lewis, L. R., Borkowski, G., Flaws, J. A.	(2020). Exposure	to di(2-ethylhexyl) phthalate and disononyl phthalate during adulthood disrupts		
Health Outcome(s) and Reported	normones and ovarian folliculogenesis inroughout the prime reproductive life of the mouse. Toxicology and Applied Pharmacology 393:114952. Reproductive/Developmental-Following 10 days of exposure at various post-dosing time points (e.g., immediately post-dosing, 3-, 6-, and 9-months post- dosing depending on the experiments) histological analysis of the follicular development in ovarian tissue samples and the sex hormone present in sera					
Health Effect(s):	(e.g., testosterone, progesterone, estradiol, FSH, and Inhibin B) from adult female mice were analyzed.					
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)					
Exposure Route:	-					
Species:	Mouse-CD-1	- [mouse]-Female				
Chemical:	Diethylhexyl	Phthalate- Parent compound				
HERO ID:	7978479	-				
Domain		Metric	Rating	Comments		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The model system used in the paper, CD-1 female mice, was appropriate for the anal- ysis of the reproductive/developmental toxicological effects of DEHP and DINP. The duration of dosing, frequency, and dosage was appropriate, and delivered in a humane and appropriate way (i.e. oral administration via pipette to the mouth). However, no measures were taken to ensure the dose being administered was in fact the dose being		

Additional	Comments:	None

Overall Quality Determination

Metric 9:

Results presentation

Medium

test) date in the study.

Medium

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Chiang, C., I phthalate du Reproductive stage (e.g., p post-dosing, live pup weig Oral-Gavage Mouse-CD-1 Diethylhexy 7978481	Lewis, L. R., Borkowski, G., Flaws, J. A. (20 ring adulthood in female mice. Reproductive e/Developmental-Post-dosing (12, 15, and 18 roestrus, estrus, metestrus/diestrus), raw num duration to begin mating and overall gestatio ghts, litter sizes, sex ratio, sex hormone levels -Duration: Short-term (>1-30 days)-1-F0- pr 1 - [mouse]-Female 1 Phthalate- Parent compound	J20). Late-life of Toxicology 93 3 months depen aber and quality onal period, fert s (e.g., testoster remating (At Pl	consequences of short-term exposure to di(2-ethylhexyl) phthalate and diisononyl :28-42. ding on the experiments) estrous cyclicity presented as percent time spent in each assessment of follicles in the ovaries of mice following varying number of months tility index, number of female mice that gave birth at various months post-dosing, rone, progesterone, estradiol, FSH, and Inhibin B) at various months post-dosing. ND 39-40 female mice were exposed for 10 days with a single oral dose/day)
Domain	mality	Metric	Rating	Comments
Domain 1: Reporting Q	Metric 1:	Reporting Quality	Medium	The test animals used were CD-1 female mice purchased from Charles Rivers (Wilmington, MA). These mice were maintained in ideal conditions regarding temperature (21.1 \pm 2.2 °C), humidity (50 \pm 20 %), access to food and water (ad libitum), number of animals per cage (3 animals/cage), and day/night cycles (12h/12hr). All procedures were approved by the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee (Protocol No.: 17079). The test chemical (DEHP) was purchased from Sigma Aldrich (St. Louis, MO), however the CASN was not provided nor was the catalog number from Sigma Aldrich. The study doses animals orally via insertion of a pipette tip into the mouth, utilizes a control (corn oil vehicle), and includes a large range of doses: 20 $\mu g/kg/day$, 200 $\mu g/kg/day$, 20 mg/kg/day, and 200 mg/kg/day. Dosing occurred at PND 39-40 for 10 days followed by various post-dosing assessments for reproduction/developmental endpoints. One of these metrics includes 12, 15, and 18-month post-exposure assessments on female mice for follicular development, cyclicity, breeding, the number of successful births, and hormone levels in sera from blood collections. Breeding occurred with untreated male mice (7-wks old) in a harem fashion (2 females per male).
Domain 2: Selection an	nd Performance			
	Metric 2:	Allocation	Low	There is no explicit language indicating use of randomization for allocating animals to groups to reduce bias in this study.
	Metric 3:	Observational Bias / Blinding Changes	High	To blind counters to treatment groups and avoid bias, ovaries were given a unique his- tological ID with no relation to treatment group. Other metrics did not state similar blinding, however, the objectivity (number of pups born) or experimental/technical con- trols for sex hormone levels in sera are considered sufficient for proper analysis.
Domain 3: Confoundin	g / Variable Co	ntrol		
	Metric 4:	Confounding / Variable Control	Medium	The study has minor confounds that may have minimally affected the results. For ex- ample, there is no indication of using randomization when assigning mice to their ex- perimental group. Also, the chemical being used is not listed with all the relevant infor- mation regarding it's purity and measures were not taken to ensure the dose given to the mice was delivered sufficiently. However, measures were taken to reduce variability and bias such as collecting all tissue and samples during the diestrus phase.

Continued on next page ...

May 2025

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 7978481 Table: 1 of 1

		cont	inued from previ	ous page		
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Chiang, C., Lewis, L. R., Borkowski, G., Flaws, J. A. (2020). Late-life consequences of short-term exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood in female mice. Reproductive Toxicology 93:28-42. Reproductive/Developmental-Post-dosing (12, 15, and 18 months depending on the experiments) estrous cyclicity presented as percent time spent in each stage (e.g., proestrus, estrus, metestrus/diestrus), raw number and quality assessment of follicles in the ovaries of mice following varying number of months post-dosing, duration to begin mating and overall gestational period, fertility index, number of female mice that gave birth at various months post-dosing. live pup weights, litter sizes, sex ratio, sex hormone levels (e.g., testosterone, progesterone, estradiol, FSH, and Inhibin B) at various months post-dosing. Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0- premating (At PND 39-40 female mice were exposed for 10 days with a single oral dose/day) Mouse-CD-1 - [mouse]-Female Diethylhexyl Phthalate- Parent compound 7978481					
Domain		Metric	Rating	Comments		
Domain 4: Selective Re	porting and Af Metric 5:	ttrition Selective Reporting and Attrition	High	There is no indication of attrition or animals being removed from the study due to health concerns. In a couple instances the authors do communicate the number of pups born to a specific dose groups were too low in number to perform statistical analysis, which can be a common occurrence when breeding mice. In these instances, the results were not statistically analyzed. Another example includes the exclusion of sex determinations from litters with cannibalized pups due to the difficulty in accurately determining sex of the pups. In these cases, the removal of such groups was warranted and allowed for more accurate/transparent analysis of the listed results.		
Domain 5. Evenance M	athada Sanaitir					
Domain 5: Exposure M	Metric 6:	Chemical administration and characterization	Medium	The chemical of interest was purchased from Sigma Aldrich (St. Louis, MO), however, neither the CASN number nor the catalog number from Sigma was indicated leaving room for speculation on the chemicals purity and composition. In a reference from the same author (HERO: 3070927) the purity of DEHP was stated to be 99%. There was no independent verification of the test substance purity, nor were there measures taken to ensure that each animal was getting their full dose. The exposure volume was determined based on body weight taken that day, indicating variable dosing volumes were possible.		
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study doses animals orally via insertion of a pipette tip into the mouth, utilizes a control (corn oil vehicle), and includes a large range of doses: $20 \ \mu g/kg/day$, $200 \ \mu g/kg/day$, $20 \ mg/kg/day$, and $200 \ mg/kg/day$. Dosing occurred at PND 39-40 for 10 days followed by various post-dosing assessments for reproduction/developmental endpoints. However, no statement indicating the time of pre-mating dosing was present. Despite these uncertainties, the dosing appears sensitive enough to induce observable changes to reproductive/developmental endpoints collected. The critical window of exposure is short-term exposure during adult-hood (i.e., sexually mature mice), with effects potentially affecting the first generation following exposure.		

Domain 6: Outcome Measures and Results Display

Continued on next page ...

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 7978481 Table: 1 of 1

continued from previous page							
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Chiang, C., Lewis, L. R., Borkowski, G., Flaws, J. A. (2020). Late-life consequences of short-term exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood in female mice. Reproductive Toxicology 93:28-42. Reproductive/Developmental-Post-dosing (12, 15, and 18 months depending on the experiments) estrous cyclicity presented as percent time spent in each stage (e.g., proestrus, estrus, metestrus/diestrus), raw number and quality assessment of follicles in the ovaries of mice following varying number of months post-dosing, duration to begin mating and overall gestational period, fertility index, number of female mice that gave birth at various months post-dosing. live pup weights, litter sizes, sex ratio, sex hormone levels (e.g., testosterone, progesterone, estradiol, FSH, and Inhibin B) at various months post-dosing. Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0- premating (At PND 39-40 female mice were exposed for 10 days with a single oral dose/day) Mouse-CD-1 - [mouse]-Female Diethylhexyl Phthalate- Parent compound 7978481						
Domain		Metric	Rating	Comments			
	Metric 8:	Endpoint sensitivity and specificity	Medium	The model system used in the paper, CD-1 female mice, was appropriate for the anal- ysis of the reproductive/developmental toxicological effects of DEHP. The duration of dosing, frequency, and dosage was appropriate, and delivered in a humane and appro- priate way (i.e. oral administration via pipette to the mouth). However, no measures were taken to ensure the dose being administered was in fact the dose being received by the mice. The studies sample size throughout the reference varies per group, with some groups having less than 3 samples, causing them to be dropped from statistical analysis. The authors are transparent in the sample sizes per experimental result. For the measure- ments of estrous cyclicity, the authors utilize vaginal lavages conducted at a consistent time of day (2hr post beginning of day cycle) and the sample size per group (DEHP vs. DINP, & 12 month vs. 15 month) was sufficient. Histological analysis of follicle devel- opment was conducted on ovarian tissues from exposed female mice with appropriate blinding to reduce bias. In most of these experiments, the sample size for some of the groups had a minimum of 3, which is considered quite low. The mating outcomes (e.g., time until mating, gestational duration, successful births, mating index, fertility index, gestational index) were described and reported appropriately with sufficient sample sizes for each group. Mice with litters decreased the sample size of some of the dose groups. For example, the percent femalepups from the 12-month post-dosing group for 200 mg/kg/day DEHP (n = 2 mice) was insufficient to perform statistical analysis. Some of the other groups for DEHP have sample sizes of 3, which is quite low. The measurement of sex hormones in sera of female mice was conducted using commercially available enzyme-linked immunosorbent assays (ELISAs) or radioimmunoassays conducted by the University of Virginia Center for Research in Reproduction Ligand Assay and Anal- ysis Core. Appropriate controls and calibrations were discussed and			
	Metric 9:	Results presentation	Medium	The data within the study were presented in an accurate and transparent manner. Al- though individual animal information is not present, the authors do use the appropriate quantification (sample sizes based on litters and not individual pups) and subsequent analysis (data was checked for normality and homogeneity of variance and further ana- lyzed via ANOVA and a 2-sided Dunnett's pot hoc test). Also, there is no clear indica- tion what the sample size per groups is, since the authors only present the sample sizes as a range and not for each dose. In cases where the sample size was insufficient, the au- thors do indicate dose group and state that statistical analysis was not conducted. Graphs depict variance as standard error bars, however, the actual SE values are not listed in the			

Continued on next page ...

figure nor within the results section.

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 7978481 Table: 1 of 1

	continued from previous page
Study Citation:	Chiang, C., Lewis, L. R., Borkowski, G., Flaws, J. A. (2020). Late-life consequences of short-term exposure to di(2-ethylhexyl) phthalate and diisononyl
	phthalate during adulthood in female mice. Reproductive Toxicology 93:28-42.
Health Outcome(s)	Reproductive/Developmental-Post-dosing (12, 15, and 18 months depending on the experiments) estrous cyclicity presented as percent time spent in each
and Reported	stage (e.g., proestrus, estrus, metestrus/diestrus), raw number and quality assessment of follicles in the ovaries of mice following varying number of months
Health Effect(s):	post-dosing, duration to begin mating and overall gestational period, fertility index, number of female mice that gave birth at various months post-dosing,
	live pup weights, litter sizes, sex ratio, sex hormone levels (e.g., testosterone, progesterone, estradiol, FSH, and Inhibin B) at various months post-dosing.

Metric

live pup weights, litter sizes, sex ratio, sex hormone levels (e.g., testosterone, progesterone, estradiol, FSH, and Inhibin B) at various months post-dosing. Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0- premating (At PND 39-40 female mice were exposed for 10 days with a single oral dose/day)

Comments

Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound

HERO ID: 7978481

Domain

Duration and Exposure Route:

Additional Comments: None

Overall Quality Determination

Medium

Rating

Study Citation:	Ganning, A	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology						
Health Outcome(s) and Reported Health Effect(s):	67(5):392-4 Hepatic/Liv transferase a	67(5):392-401. Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl- transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)						
Duration and	Oral-Diet-D	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)						
Exposure Route: Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 679540	e-Dawley - [rat]-Male l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Reporting (Quality Metric 1:	Reporting Quality	Medium	Critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) was reported; however, no additional details were provided. Neither the number of animals per group or the sample size was specified.				
Domain 2: Selection an	nd Performance							
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.				
Domain 3: Confoundin	ng / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group fed diets in the absence of test substance; however, it does not appear that control animals were included during the post-exposure period. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study.				
Domain 4 [.] Selective R	eporting and At	trition						
	Metric 5:	Selective Reporting and Attrition	Low	Insufficient information was provided to determined attrition or selective reporting.				
Domain 5: Exposure N	lethods Sensitiv	vity						
	Metric 6:	Chemical administration and characterization	Uninformative	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake nor body weights were measured and reliable doses in mg/kg-day cannot be determined.				
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure timing and frequency were reported. Animals were exposed via the diet for 2 weeks. No justification was provided by the study author.				
		Co	ntinued on next page .					

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 679540 Table: 1 of 1

			continued from previous p	age			
Study Citation:	Ganning, A.	E., Olsson, M. J., Brunk, U., Dallner, G. (1	1990). Effects of prolonged	treatment with phthalate ester on rat liver. Pharmacology & Toxicology			
Health Outcome(s) and Reported	67(5):392-4 Hepatic/Live transferase a	67(5):392-401. Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl- transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)					
Health Effect(s): Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)						
Species: Chemical: HERO ID:	Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound 679540						
Domain		Metric	Rating	Comments			
Domain 6: Outcome Mo	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	The study included a single endpoint (measurement of hepatic enzyme activities). It is unclear what the purpose of the study was. Enzyme activities alone are not considered to be a sensitive endpoint for assessing hepatic toxicity. No methodological details were provided, although similar measurements were reported for another experiment reported in the same study. Methods of enzyme measurements were cited to other sources.			
	Metric 9:	Results presentation	Uninformative	Results were qualitatively described in the study text. An increase in enzyme activity was mentioned; however, no statistical analysis was done and no data were provided to conduct an independent analysis.			
Additional Comments:	None						
Overall Quali	ty Detern	nination	Uninformative				

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520. Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testos- terone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro. Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s) Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 674162					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); the CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection an	d Performance					
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.		
	Metric 3:	Observational Bias / Blinding Changes	High	The study reports "observers were blinded to treatment condition to avoid bias". It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.		
Domain 3: Confounding	g / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether ap- proaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the pres- ence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dis- pensing containers were not described.		

HERO ID: 674162 Table: 1 of 4

		conti	nued from previ	ious page			
Study Citation:	Ge, R. S., C	hen, G. R., Dong, Q., Akingbemi, B., Sottas	, C. M., Santos, N	M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal Journal of Andrology 28(4):513-520			
Health Outcome(s) and Reported	exposure to dietnyinexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520. Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testos terone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro. Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)						
Duration and							
Exposure Route:							
Species: Chemical:	Diethvlhexy	vans - [rat]-Male vl Phthalate- Parent compound					
HERO ID:	674162	·····					
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for most outcomes in tabular form and in the text. The study states that the experiment was ran twice with 10 males/group each time. Data for both trials were combined; therefore there should be data for 20 males/group. Table 1 reports the number of animals as n= 40 (control group); n=19 (for the 10 and 500 mg/kg/day groups); and n=25 (for the 750 mg/kg/day group). It is unclear where the extra animals came from. The authors did not report death, so it is not clear if animals in the 10 and 500 mg/kg/day group died or were not included in analysis for some other reason. Seminal vesicle weights in the 750 mg/kg/day group were not determined; no explanation for this is given.			
Domain 5: Exposure M	Iethods Sensiti	vity					
·	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if study authors adjusted for weight changes when delivering the test substance.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest. The authors state "This age interval was used because the prepubertal period is a time of active reproductive tract development, and hormonally active chemicals are known to exhibit greater potency during sexual differentiation in rodents and humans than at later times."			
Domain 6: Outcome M	laggurga and Da	noulta Dianlay					
Domain 6: Outcome M	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm SEM. Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						
Overall Ouali	tv Deter	mination	Medium				
Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z							

|--|

Study Citation:	Ge, R. S., Ch	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal					
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testos- terone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro.						
Duration and	Oral-Gavage	-Duration: Short-term (>1-30 days)-7-14-da	ay(s)				
Species: Chemical: HERO ID:	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 674162						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); A CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	d Performance Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Low	Animals were randomly allocated to study groups based on body weights. The study reports that "observers were blinded to treatment condition to avoid bias". It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.			
Domain 3: Confounding	g / Variable Cor Metric 4:	ntrol Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether ap- proaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the pres- ence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dis- pensing containers were not described.			
Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	Low	Data were reported for all outcomes in tabular form and in the text. The methods do not report how many animals were exposed/group; but based on the 28-day experiment reported in this paper, it is reasonable to assume it may be 10/group. Data were reported for 10 animals/group. The methods specify that rats were exposed to the 500 mg/kg/day dose only for 14 days; however, in the Results (Table 2), data is presented for 10 mg/kg/day and 500 mg/kg/day. It is unclear if the 10 mg/kg/day animals were exposed concurrently or if these data came from an earlier experiment discussed by the authors.			
Domain 5: Exposure M	ethods Sensitiv	ity					

Diethylhexyl Phthalate

HERO ID: 674162 Table: 2 of 4

		cont	tinued from previo	us page		
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exmanue Route	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520. Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testos-terone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro. Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)					
Species:	Rat-Long-E	vans - [rat]-Male				
Chemical: HERO ID:	Diethylhexy 674162	l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and duration	Low High	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if the study authors adjusted for weight changes when delivering the test substance. The timing and duration of exposure were appropriate for the outcomes of interest. The study authors wanted to determine whether "compensatory changes in androgen synthesis and feedback suppression of pituitary function were associated with 28-day exposure, so a shorter duration (14-days) was assessed.		
Domain 6: Outcome Me	easures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.		
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm SEM. Statistical analysis methods were reported and appropriate.		
Additional Comments:	None					
Overall Quali	ty Deteri	mination	Medium			

Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal					
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species:	exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520. Nutritional/Metabolic-Body weight Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)					
Chemical:	Diethylhexyl	Phthalate- Parent compound				
HERO ID:	674162					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); the CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance					
	Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High High	Animals were randomly allocated to study groups based on body weights. The study reports "observers were blinded to treatment condition to avoid bias". It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.		
Domain 2: Confounding	r / Variabla Cor	atral				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether ap- proaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the pres- ence of contaminants, such as phthalates, but this is not expected to impact the ability to interpret the study results for the selected endpoints. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.		
Domain 1: Selective Re	porting and Att	rition				
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for most outcomes in tabular form and in the text. The study states that the experiment was ran twice with 10 males/group each time. Data for both trials were combined; therefore there should be data for 20 males/group. Table 1 reports the number of animals as $n=40$ (control group); $n=19$ (for the 10 and 500 mg/kg/day groups); and $n=25$ (for the 750 mg/kg/day group). It is unclear where the extra animals came from. The authors did not report death, so it is not clear if animals in the 10 and 500 mg/kg/day group died or were not included in analysis for some other reason. Seminal vesicle weights in the 750 mg/kg/day group were not determined; no explanation for this is given.		

Diethylhexyl Phthalate

		conti	nued from previ	ious page			
Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal av posure to disthulbeavelableate on the timing of puberty in male rate. Journal of Andrology 28(4):513–520						
Health Outcome(s) and Reported	Nutritional/Metabolic-Body weight						
Health Effect(s):							
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-/-28-0	day(s)				
Exposure Route:	Dat Long E	vong [rot] Mala					
Species: Chomical:	Diethylbey	Valls - [lat]-Walc					
HERO ID:	674162	a r nulaiate- r arent compound					
Domain	071102	Matria	Dating	Commonts			
Domain 5: Exposure M	lethods Sensitiv	vity	Katilig	Comments			
Domain 5. Exposure i	Metric 6:	Chemical administration and	Low	The source and purity of the test substance were not reported. Gayage volume was not			
		characterization	2011	reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if study authors adjusted for weight changes when delivering the test substance.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest. The authors state "This age interval was used because the prepubertal period is a time of active reproductive tract development, and hormonally active chemicals are known to exhibit greater potency during sexual differentiation in rodents and humans than at later times."			
Domain 6: Outcome M	leasures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm SEM. Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						

Overall Quality Determination

Medium

Study Citation:	Ge, R. S., C exposure to Nutritional/	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.					
and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Gavag Rat-Long-E Diethylhexy 674162	e-Duration: Short-term (>1-30 days)-7-14-da wans - [rat]-Male yl Phthalate- Parent compound	ıy(s)				
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); A CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection an	d Performance Metric 2: Metric 3:	e Allocation Observational Bias / Blinding Changes	High Low	Animals were randomly allocated to study groups based on body weights. The study reports that "observers were blinded to treatment condition to avoid bias". It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.			
Domain 3: Confoundin	g / Variable Co Metric 4:	ontrol Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether ap- proaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the pres- ence of contaminants, such as phthalates, but this is not expected to impact the ability to interpret the study results for the selected endpoints. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.			
Domain 4: Selective Re	porting and A Metric 5:	ttrition Selective Reporting and Attrition	Low	Data were reported for all outcomes in tabular form and in the text. The methods do not report how many animals were exposed/group; but based on the 28-day experiment reported in this paper, it is reasonable to assume it may be 10/group. Data were reported for 10 animals/group. The methods specify that rats were exposed to the 500 mg/kg/day dose only for 14 days; however, in the Results (Table 2), data is presented for 10 mg/kg/day and 500 mg/kg/day. It is unclear if the 10 mg/kg/day animals were exposed concurrently or if these data came from an earlier experiment discussed by the authors.			
Domain 5: Exposure M	ethods Sensiti	vity					
		Contin	ued on next pa	ıge			

		cont	inued from previo	bus page			
Study Citation:	Ge, R. S., Ch	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal					
Health Outcome(s)	Nutritional/Metabolic-Body weight						
and Reported Health Effect(s):							
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)						
Exposure Route:	Dat Lang E						
Species: Chemical:	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound						
HERO ID:	674162						
Domain		Metric	Rating	Comments			
	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and duration	Low High	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if the study authors adjusted for weight changes when delivering the test substance. The timing and duration of exposure were appropriate for the outcomes of interest. The study authors wanted to determine whether "compensatory changes in androgen synthesis and feedback suppression of pituitary function were associated with 28-day exposure, so a shorter duration (14-days) was assessed.			
Domain 6: Outcome Me	asures and Rea	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm SEM. Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						
Overall Qualit	y Detern	nination	Medium				

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	Grande, S. di(2-ethylhe Reproductiv clinical sign nipples/areo evaluated da opening to d Oral-Gavage Rat-Wistar - Diethylhexy	W., Andrade, A. J., Talsness, C. E., Grote, xyl)phthalate: effects on female rat reproduct e/Developmental-Ovary weightLitter size, set s, were assessed; on PND1 brain and liver w las. On PND 22, measurement of anogenital ily for vaginal opening. Body weights were r letect first day of estrusNutritional/Metaboli e-Duration: Short-term (>1-30 days)-1-F0 - g [rat]-Female l Phthalate- Parent compound	K., Chahoud, I tive development x ratio, pup wei veights measure distance (AGE measured on da c-Body weight gestation (GD6-	. (2006). A dose-response study following in utero and lactational exposure to nt. Toxicological Sciences 91(1):247-254. ight, post implantation loss and number of viable pups were assessed. In offspring: dd (1-2 females/litter); PND13, all female pups were examined for the number of 0) and brain and liver weights. Beginning on PND33, all remaining females were y of vaginal opening. Daily vaginal smears were assessed from the day of vaginal of dams to birth)-F0- lactation (birth-PND21)
HERO ID:	674171			
Domain 1: Reporting Or	uality	Metric	Rating	Comments
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and	d Performance			
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.
Domain 3: Confounding	/ Variable Co	ntrol		
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Rep	porting and At	trition		
		Contin	ued on next pa	ge

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 674171 Table: 1 of 8

		conti	nued from prev	ious page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Grande, S. di(2-ethylhe Reproductiv clinical sign nipples/areo evaluated da opening to c Oral-Gavago Rat-Wistar - Diethylhexy 674171	W., Andrade, A. J., Talsness, C. E., Grote exyl)phthalate: effects on female rat reprodu re/Developmental-Ovary weightLitter size, s ss, were assessed; on PND1 brain and liver blas. On PND 22, measurement of anogenit uily for vaginal opening. Body weights were letect first day of estrusNutritional/Metabo e-Duration: Short-term (>1-30 days)-1-F0 - [rat]-Female black-Parent compound	 K., Chahoud, ictive developme sex ratio, pup we weights measur al distance (AGI e measured on da olic-Body weight gestation (GD6 	I. (2006). A dose-response study following in utero and lactational exposure to ent. Toxicological Sciences 91(1):247-254. eight, post implantation loss and number of viable pups were assessed. In offspring: ed (1-2 females/litter); PND13, all female pups were examined for the number of D) and brain and liver weights. Beginning on PND33, all remaining females were ay of vaginal opening. Daily vaginal smears were assessed from the day of vaginal t of dams -to birth)-F0- lactation (birth-PND21)
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure N	lathada Sanaitir	it.		
Domain 5: Exposure M	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and duration	Low High	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 67367). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels. This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriate for other endpoints examined given the aim of the study.
Demain (+ Outer - M	(lta Di las		
	Metric 8:	Endpoint sensitivity and specificity	High	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined. Endpoints examined were sensitive to assess developmental, body weight and clinical sign endpoints.
		Conti	inued on next p	age

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HERO ID: 674171 Table: 1 of 8

			continued from previo	us page	
Study Citation:	Grande, S. W	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to			
Health Outcome(s) and Reported Health Effect(s):	di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254. Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed. In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal				
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)				
Exposure Route:					
Species:	Rat-Wistar -	[rat]-Female			
Chemical:	Diethylhexyl	Phthalate- Parent compound			
HERO ID:	674171				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	High	This study is considered High for Metric 6.2. Results were fully reported. Data was presented as means +/- variance and number of animal or litters examined. Statistical analysis was appropriate. Normality and homogeneity of variances were evaluated prior to data analysis. The study used a linear mixed model (proc mixed) with treatment as a main effect and litter as a random factor (nested for treatment) to adjust for litter effects. Organ weights and AGD were analyzed with body weight as a covariate.	
Additional Comments:	None				
Overall Quali	ty Detern	nination	Medium		

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Grande, S. di(2-ethylhe Reproductiv clinical sigr nipples/arec evaluated da opening to c Oral-Gavag Rat-Wistar Diethylhexy 674171	W., Andrade, A. J., Talsness, C. E., Grote, exyl)phthalate: effects on female rat reproduct re/Developmental-Ovary weightLitter size, se us, were assessed; on PND1 brain and liver w blas. On PND 22, measurement of anogenital aily for vaginal opening. Body weights were r letect first day of estrusNutritional/Metaboli e-Duration: Short-term (>1-30 days)-1-F0 - g [rat]-Female 1 Phthalate- Parent compound	K., Chahoud, I ive developmer x ratio, pup wei veights measure distance (AGD neasured on da c-Body weight gestation (GD6-	. (2006). A dose-response study following in utero and lactational exposure to nt. Toxicological Sciences 91(1):247-254. ight, post implantation loss and number of viable pups were assessed. In offspring: ed (1-2 females/litter); PND13, all female pups were examined for the number of 0) and brain and liver weights. Beginning on PND33, all remaining females were y of vaginal opening. Daily vaginal smears were assessed from the day of vaginal of dams to birth)-F0- lactation (birth-PND21)	
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.	
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.	
Domain 3: Confounding	g / Variable Cc Metric 4:	ntrol Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.	
Domain 4: Selective Re	porting and At	trition			
Continued on next page					

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

		cont	tinued from previ	ious page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Grande, S. V di(2-ethylhe: Reproductive clinical sign: nipples/areol evaluated da opening to d Oral-Gavage Rat-Wistar - Diethylhexy 674171	W., Andrade, A. J., Talsness, C. E., Grot xyl)phthalate: effects on female rat reprod e/Developmental-Ovary weightLitter size, s, were assessed; on PND1 brain and live las. On PND 22, measurement of anogen ily for vaginal opening. Body weights we etect first day of estrusNutritional/Metat e-Duration: Short-term (>1-30 days)-1-FO [rat]-Female l Phthalate- Parent compound	te, K., Chahoud, I luctive developmen , sex ratio, pup we er weights measure ital distance (AGE re measured on da polic-Body weight) - gestation (GD6-	I. (2006). A dose-response study following in utero and lactational exposure to nt. Toxicological Sciences 91(1):247-254. ight, post implantation loss and number of viable pups were assessed. In offspring: ed (1-2 females/litter); PND13, all female pups were examined for the number of D) and brain and liver weights. Beginning on PND33, all remaining females were y of vaginal opening. Daily vaginal smears were assessed from the day of vaginal of dams -to birth)-F0- lactation (birth-PND21)
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from $n=11-16$ in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day $n=14$, whereas the other organ weights had $n=15$). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure M	ethods Sensitiv	ity		
Domain 5. Exposure int	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and duration	Low High	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on stor- age conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels. This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for the study diven the acim of the study.
				aso appropriate for other endpoints examined given the ann of the study.
Domain 6: Outcome Me	easures and Res Metric 8:	sults Display Endpoint sensitivity and specificity	High	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined. Endpoints examined were sensitive to assess developmental, body weight and clinical sign endpoints.
		Con	tinued on next pa	nge

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HERO ID: 674171 Table: 2 of 8

			continued from previo	us page	
Study Citation:	Grande, S. W	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to			
Health Outcome(s) and Reported Health Effect(s):	di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254. Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed. In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal				
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)				
Exposure Route:					
Species:	Rat-Wistar -	[rat]-Female			
Chemical:	Diethylhexyl	Phthalate- Parent compound			
HERO ID:	674171				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	High	This study is considered High for Metric 6.2. Results were fully reported. Data was presented as means +/- variance and number of animal or litters examined. Statistical analysis was appropriate. Normality and homogeneity of variances were evaluated prior to data analysis. The study used a linear mixed model (proc mixed) with treatment as a main effect and litter as a random factor (nested for treatment) to adjust for litter effects. Organ weights and AGD were analyzed with body weight as a covariate.	
Additional Comments:	None				
Overall Quali	ty Detern	nination	Medium		

Study Citation:	Grande, S. V di(2-ethylbe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.					
Health Outcome(s) and Reported	 (a) Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Splee and thymus weight Coral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21) 						
Duration and							
Exposure Route:	Dot Wistor	Det Wister Fret Formele					
Chemical:	Diethylhexy	.at-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound					
HERO ID:	674171	-					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.			
Domain 3. Confounding	y / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.			
Domain 4: Selective Re	porting and At	trition					

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674171 Table: 3 of 8

		conti	inued from previ	ious page		
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254. Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight					
Duration and Exposure Route:	Oral-Gavag	e-Duration: Short-term (>1-30 days)-1-F0	- gestation (GD6	-to birth)-F0- lactation (birth-PND21)		
Species: Chemical: HERO ID:	Rat-Wistar Diethylhexy 674171	- [rat]-Female /l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.		
Domain 5: Exposure N	lathods Sansitiv					
Domain 5: Exposure M	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.		
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.		
Domain 6: Outcome M	leasures and Re	esults Disnlav				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.		

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Diethylhexyl Phthalate

			. continued from previ	ous page
Study Citation:	Grande, S. di(2-ethylhe	W., Andrade, A. J., Talsness, C. E. exvl)phthalate: effects on female rat 1	, Grote, K., Chahoud, I reproductive development	. (2006). A dose-response study following in utero and lactational exposure to tt. Toxicological Sciences 91(1):247-254.
Health Outcome(s)	Neurologica	al/Behavioral-Brain weight-Hepatic/I	Liver-Liver weight-Rena	/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen
and Reported	and thymus	weight		
Health Effect(s):				
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)	-1-F0 - gestation (GD6-	to birth)-F0- lactation (birth-PND21)
Exposure Route:				
Species:	Rat-Wistar	- [rat]-Female		
Chemical:	Diethylhexy	Phthalate- Parent compound		
HERO ID:	674171			
Domain		Metric	Rating	Comments
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.
Additional Comments:	None			

Overall Quality Determination

Medium

Study Citation:	Grande, S. V di(2-ethylhe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.					
Health Outcome(s) and Reported Health Effect(s):	Neurologica and thymus	l/Behavioral-Brain weight-Hepatic/Liver-Liv weight	-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen				
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)						
Exposure Koute: Species:	Rat-Wistar -	Rat-Wistar - [rat]-Female					
Chemical:	Diethylhexy	Jiethylhexyl Phthalate- Parent compound					
HERO ID:	674171						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported.			
				sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mat- ing). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.			
Domain 3: Confounding	y / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.			
Domain 4: Selective Re	porting and At	trition					

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674171 Table: 4 of 8

		-					
Grande, S. di(2-ethylhe	W., Andrade, A. J., Talsness, C. E., Grote xyl)phthalate: effects on female rat reprodu	, K., Chahoud, I ctive developme	. (2006). A dose-response study following in utero and lactational exposure to nt. Toxicological Sciences 91(1):247-254.				
Neurologica	l/Behavioral-Brain weight-Hepatic/Liver-L	iver weight-Rena	l/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen				
and thymus	weight						
Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)							
Rat-Wistar - [rat]-Female							
Diethylhexy	I Phthalate- Parent compound						
6/41/1							
	Metric	Rating	Comments				
Meine 3:	Selective Reporting and Aurition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from $n=11-16$ in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day $n= 14$, whereas the other organ weights had $n=15$). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.				
lethods Sensitiv	/ity	-					
Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on stor- age conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.				
Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.				
anguras and D-	sulta Display						
easures and Re Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.				
	Grande, S. di(2-ethylhe Neurologica and thymus Oral-Gavage Rat-Wistar - Diethylhexy 674171 Metric 5: Iethods Sensitiv Metric 6: Metric 7: easures and Re Metric 8:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote di(2-ethylhexyl)phthalate: effects on female rat reprodu Neurological/Behavioral-Brain weight-Hepatic/Liver-L and thymus weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 674171 <u>Metric</u> Metric 5: Selective Reporting and Attrition lethods Sensitivity Metric 6: Chemical administration and characterization Metric 7: Exposure timing, frequency, and duration easures and Results Display Metric 8: Endpoint sensitivity and specificity	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I di(2-ethylhexyl)phthalate: effects on female rat reproductive developmen Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Rena and thymus weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6- Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 674171 Metric Rating Metric 5: Selective Reporting and Attrition Low characterization Metric 6: Chemical administration and characterization Metric 7: Exposure timing, frequency, and High duration Metric 8: Endpoint sensitivity and specificity Metric 8: Endpoint sensitivity and specificity				

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Diethylhexyl Phthalate

			. continued from previ	ous page		
Study Citation:	Grande, S. di(2-ethylhe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2, ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254				
Health Outcome(s)	Neurologica	l/Behavioral-Brain weight-Hepatic/L	iver-Liver weight-Rena	l/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen		
and Reported	and thymus	weight				
Health Effect(s):						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)	-1-F0 - gestation (GD6-	to birth)-F0- lactation (birth-PND21)		
Exposure Route:						
Species:	Rat-Wistar ·	- [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674171					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation:	Grande, S. V	W., Andrade, A. J., Talsness, C. E., Grote, Xul)phthalate: effects on female rat reproduct	K., Chahoud, I	. (2006). A dose-response study following in utero and lactational exposure to				
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight							
Duration and	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)						
Exposure Route:	D (W ^r (
Species: Chemical:	Diethylhexy	[rat]-remaie] Phthalate- Parent compound						
HERO ID:	674171							
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality							
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.				
Domain 2: Selection and	d Performance							
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.				
Domain 3: Confounding	y / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.				
Domain 4: Selective Re	porting and At	trition						

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674171 Table: 5 of 8

		сопи	nued from previ	ious page			
Study Citation:	Grande, S. di(2-ethylhe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.					
and Reported	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Sp and thymus weight						
Health Effect(s):							
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-F0	- gestation (GD6-	-to birth)-F0- lactation (birth-PND21)			
Exposure Route:							
Species:	Rat-Wistar -	[rat]-Female					
Chemical:	Diethylhexy	I Phthalate- Parent compound					
	0/41/1						
Domain	Matria 5.	Metric	Rating	Comments			
				dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from $n=11-16$ in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day $n= 14$, whereas the other organ weights had $n=15$). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.			
Domain 5: Exposure M	athods Sansitiz	zity					
Domain 5: Exposure M	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.			
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.			
Domain 6: Outcome M	ancurac and Da	culte Dieploy					
Domain 0. Outcome M	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses			
				were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.			

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Diethylhexyl Phthalate

			. continued from previ	ous page		
Study Citation:	Grande, S. di(2-ethylhe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylbeyyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254				
Health Outcome(s)	Neurologica	al/Behavioral-Brain weight-Hepatic/I	Liver-Liver weight-Rena	/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen		
and Reported	and thymus	weight				
Health Effect(s):						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)	-1-F0 - gestation (GD6-	to birth)-F0- lactation (birth-PND21)		
Exposure Route:						
Species:	Rat-Wistar	- [rat]-Female				
Chemical:	Diethylhexy	Phthalate- Parent compound				
HERO ID:	674171					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation:	Grande, S. V di(2-ethylbe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.						
Health Outcome(s) and Reported Health Effect(s):	Neurologica and thymus	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight						
Duration and Exposure Route:	Oral-Gavage	Dral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)						
Species:	Rat-Wistar -	Rat-Wistar - [rat]-Female						
Chemical: HERO ID:	Diethylhexy 674171	l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality Matria 1:	Poporting Quality	Madium	This study is considered Medium for Matrie 1. Test shaming was described as DEUD				
	Mether 1:	Reporting Quanty	Medium	Inis study is considered Medulin for Metric 1. Test chemical was described as DEFIF, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mat- ing). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.				
Domain 2: Selection and	d Performance							
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.				
Domain 3: Confounding	y / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.				
Domain 4: Selective Re	porting and At	trition						

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674171 Table: 6 of 8

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Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.					
Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Sple and thymus weight					
Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)					
Pat-Wistor	[rat]_Female				
Diethylhexy	Phthalate- Parent compound				
674171					
	Metric	Rating	Comments		
Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.		
athada Sanaitir					
ethods Sensitiv Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.		
Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.		
easures and De	sults Display				
Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed histological evaluation would have provided more information		
	Grande, S. di(2-ethylhe Neurologica and thymus Oral-Gavage Rat-Wistar - Diethylhexy 674171 Metric 5: ethods Sensitiv Metric 6: Metric 7: easures and Re Metric 8:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote di(2-ethylhexyl)phthalate: effects on female rat reprodu Neurological/Behavioral-Brain weight-Hepatic/Liver-L and thymus weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 674171 Metric Metric 5: Selective Reporting and Attrition ethods Sensitivity Metric 5: Chemical administration and characterization Metric 7: Exposure timing, frequency, and duration easures and Results Display Metric 8: Endpoint sensitivity and specificity	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, di(2-ethylhexyl)phthalate: effects on female rat reproductive developme Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renz and thymus weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6 Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 674171 <u>Metric Rating</u> Metric 5: Selective Reporting and Attrition Low ethods Sensitivity Metric 6: Chemical administration and Low characterization Metric 7: Exposure timing, frequency, and High duration High Metric 8: Endpoint sensitivity and specificity Medium		

Continued on next page ...

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Diethylhexyl Phthalate

			. continued from previ	ous page		
Study Citation:	Grande, S.	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to				
Health Outcome(s)	Neurologica	ll/Behavioral-Brain weight-Hepatic/L	liver-Liver weight-Rena	/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen		
and Reported	and thymus	weight				
Health Effect(s):						
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)	-1-F0 - gestation (GD6-	to birth)-F0- lactation (birth-PND21)		
Exposure Route:						
Species:	Rat-Wistar -	· [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674171					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation:	Grande, S. V	W., Andrade, A. J., Talsness, C. E., Grote,	K., Chahoud, I	. (2006). A dose-response study following in utero and lactational exposure to				
Health Outcome(s) and Reported Health Effect(s):	Neurologica and thymus	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight						
Duration and	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)						
Exposure Route:	DIN							
Species: Chemical:	Rat-Wistar - Diethylhexy	Rat-Wistar - [rat]-Female						
HERO ID:	674171							
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality							
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.				
Domain 2: Selection and	d Performance							
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.				
Domain 3: Confounding	y / Variable Co	ntrol						
	Metric 4:	 / Variable Control Metric 4: Confounding / Variable Control Medium This study is considered Medium for Metric 3. A concurrent appropriate negative con trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected be minimal and reported information did not identify differences among study groups 						
Domain 4: Selective Re	porting and At	trition						

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674171 Table: 7 of 8

		-			
Grande, S. di(2-ethylhe	W., Andrade, A. J., Talsness, C. E., Grote xyl)phthalate: effects on female rat reprodu	, K., Chahoud, I ctive developme	. (2006). A dose-response study following in utero and lactational exposure to nt. Toxicological Sciences 91(1):247-254.		
Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Splee and thymus weight					
Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)					
Diethylhexy	I Phthalate- Parent compound				
6/41/1					
	Metric	Rating	Comments		
Meine 3:	Selective Reporting and Aurition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from $n=11-16$ in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day $n= 14$, whereas the other organ weights had $n=15$). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.		
lethods Sensitiv	/ity	-			
Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on stor- age conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.		
Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.		
anguras and D-	sulta Display				
easures and Re Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.		
	Grande, S. di(2-ethylhe Neurologica and thymus Oral-Gavage Rat-Wistar - Diethylhexy 674171 Metric 5: Iethods Sensitiv Metric 6: Metric 7: easures and Re Metric 8:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote di(2-ethylhexyl)phthalate: effects on female rat reprodu Neurological/Behavioral-Brain weight-Hepatic/Liver-L and thymus weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 674171 <u>Metric</u> Metric 5: Selective Reporting and Attrition lethods Sensitivity Metric 6: Chemical administration and characterization Metric 7: Exposure timing, frequency, and duration easures and Results Display Metric 8: Endpoint sensitivity and specificity	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I di(2-ethylhexyl)phthalate: effects on female rat reproductive developmen Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Rena and thymus weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6- Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 674171 Metric Rating Metric 5: Selective Reporting and Attrition Low characterization Metric 6: Chemical administration and characterization Metric 7: Exposure timing, frequency, and High duration Metric 8: Endpoint sensitivity and specificity Metric 8: Endpoint sensitivity and specificity		

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Diethylhexyl Phthalate

			. continued from previ	ous page		
Study Citation:	Grande, S. di(2-ethylhe	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2, ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254				
Health Outcome(s)	Neurologica	l/Behavioral-Brain weight-Hepatic/L	iver-Liver weight-Rena	l/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen		
and Reported	and thymus	weight				
Health Effect(s):						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)	-1-F0 - gestation (GD6-	to birth)-F0- lactation (birth-PND21)		
Exposure Route:						
Species:	Rat-Wistar ·	- [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674171					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation: Health Outcome(s)	Grande, S. V di(2-ethylhe Other (pleas	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254. Other (please specify below) (Clinical signs)-Clinical signs of toxicity						
and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Gavage Rat-Wistar - Diethylhexy 674171	Dral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21) Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 574171						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mat- ing). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.				
Domain 2: Selection and	d Performance Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly as- signed to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nip- ples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not ex- pected to have a substantial impact on results.				
Domain 3: Confounding	v / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative con- trol was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.				
Domain 4: Selective Rep	porting and At	trition						

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HERO ID: 674171 Table: 8 of 8

		conti	nued from previ	ious page			
Study Citation: Health Outcome(s) and Reported	Grande, S. di(2-ethylhe Other (pleas	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254. Other (please specify below) (Clinical signs)-Clinical signs of toxicity					
Health Effect(s): Duration and Exposure Route: Species: Chemical:	Oral-Gavag Rat-Wistar - Diethylhexy	e-Duration: Short-term (>1-30 days)-1-F0 · [rat]-Female ·l Phthalate- Parent compound	- gestation (GD6-	-to birth)-F0- lactation (birth-PND21)			
HERO ID:	674171	-					
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.			
Domain 5: Exposure M	lethods Sensitiv	vity					
Domain 5. Exposule iv	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.			
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.			
Domain 6: Outcome M	easures and Re	sults Disnlav					
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined. Details regarding assessment of clinical signs are lacking. Animals were assessed daily, however, characteristics or behavior the authors would have considered adverse were not provided.			
		Cont	inued on next pa	age			

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continued from previous page							
Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to						
Health Outcome(s)	di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254. Other (please specify below) (Clinical signs)-Clinical signs of toxicity						
and Reported Health Effect(s):							
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)						
Species:	Rat-Wistar - [rat]-Female						
Chemical: HERO ID:	Diethylhexyl Phthalate- Parent compound 674171						
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Clinical signs were reported as negative in the text.			
Additional Comments:	None						
Overall Qualit	y Deterr	mination	Medium				

Study Citation	Grav I. Ba	rlow N. Howdechell K. Osthy I. Eurr I.	Grav C (20	000) Transgenerational effects of Di (2 athylbeyyl) phthalate in the male CPL (CD(SD)			
Health Outcome(s) and Reported	 Gray, L., Barlow, N., Howdesnell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CR rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain 						
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND						
Species: Chemical: HERO ID:	Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697475						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	Quality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection an	nd Performance						
	Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Medium	Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).			
Domain 3: Confoundin	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the pres- ence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. These lack of details are not expected to significantly impact the endpoints described.			
Domain 4: Selective Re	eporting and At	trition					
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.			
Domain 5: Exposure Methods Sensitivity							
Continued on next page							
May 2025 Human Health Hazard Animal Toxicology Evaluation

continued from previous page							
Study Citation: Health Outcome(s)	Gray, L., Ba rat: Added Mortality-M	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain					
and Reported Health Effect(s): Duration and Exposure Route:	Oral-Gavag	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)					
Species:	Rat-Sprague	e-Dawley - [rat]-Female					
Chemical: HERO ID:	Diethylhexyl Phthalate- Parent compound 697475						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.			
Domain 6: Outcome Me	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups			
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm standard error. Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						
Overall Quality Determination		High					

Study Citation:	Gray, L., Bar rat: Added v	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425					
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mo	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain					
Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)					
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexyl 697475	-Dawley - [rat]-Female Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	d Performance Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Medium	Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).			
Domain 3: Confounding	g / Variable Cor	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the pres- ence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. These lack of details are not expected to significantly impact the endpoints described.			
Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.			
Domain 5: Exposure M	ethods Sensitiv	ity					
Continued on next page							

		cont	inued from p	revious page			
Study Citation:	Gray, L., Ba rat: Added	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.					
and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain						
Duration and Exposure Route:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-F0	- gestation (C	GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)			
Species:	Rat-Sprague	e-Dawley - [rat]-Female					
Chemical: HERO ID:	Diethylhexy 697475	Diethylhexyl Phthalate- Parent compound					
Domain	0,7110	Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.			
Domain 6: Outcome Me	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm standard error. Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						
Overall Quality Determination			High				

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides. Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65) Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697475				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CASRN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Animals were pregnant on purchase but parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance Metric 2:	Allocation	High	Pregnant dams were randomly allocated to study groups based on body weight. Male pups were randomly selected from litter for the PUB cohort; the method was not re-	
	Metric 3:	Observational Bias / Blinding Changes	High	Assessors were blinded to treatment groups when assessing AGD and determining are- ola/nipple numbers and location. The study does not indicate if other endpoints were assessed blindly, although most were non-subjective (body and organ weights) or initial histopathological examination.	
Domain 2. Confounding	Variable Ca	ateol			
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with stirring bar removed to avoid contact with plastics.	
Domain 4: Selective Re	norting and Δt	rition			
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for most outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block (two blocks included). Results are shown for 13-14 dams/group. The number of male offspring examined is reported in the results tables. There is no indication that any animals were excluded from the analysis.	
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		conti	inued from previ	ous page		
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides. Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65) Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697475					
Domain		Metric	Rating	Comments		
Domain 5: Exposure M	lethods Sensitiv Metric 6: Metric 7:	vity Chemical administration and characterization Exposure timing, frequency, and	Medium High	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are pro- vided, and doses were not analytically verified. The timing and duration of exposure were appropriate for the outcomes of interest and		
		duration		were justified by the study authors.		
Domain 6: Outcome M	easures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. Based on the power analysis conducted by the authors, the number of males analyzed in this cohort was insufficient and lacked the statistical power to adequately detect reproductive histopathological changes. Doses chosen were similar to those previously reported findings in the literature. The methods of endpoint assessments were adequately described, appropriate, and sensitive to the outcomes of interest. Outcomes were assessed consistently across study groups. The test species and strain were appropriate and justified by the study authors.		
	Metric 9:	Results presentation	Low	Results were described in the text and data were presented in tables as means \pm standard error. Statistical analysis methods were reported and were appropriate in some cases; the litter was used as the statistical unit. Histopathology and gross malformation data for the PUB cohort alone were not adequately reported. Histopathology data were inappropriately combined and analyzed (across study cohorts and blocks, from two exposure durations), which significantly impacts the ability to interpret the study results. Individual animal data were not provided.		
Additional Comments:	None					
Overall Quali	ty Deteri	mination	Medium			

Study Citation:	Gray, L., Bar rat: Added y	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Other (please specify below) (Clinical signs)-Observational health of dams					
Health Outcome(s) and Reported Health Effect(s):	Other (please						
Duration and	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)					
Exposure Route:	Rat-Sprague.	Dawley - [rat]-Female					
Chemical: HERO ID:	Diethylhexyl 697475	Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	d Performance						
	Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Medium	Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported for clinical signs.			
Domain 3 [.] Confounding	y / Variable Cor	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the pres- ence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.			
Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.			
Domain 5: Exposure M	ethods Sensitivi	ity					
		Contin	ued on next pa				

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		cont	inued from previo	us page			
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Gray, L., Ba rat: Added v Other (pleas	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Other (please specify below) (Clinical signs)-Observational health of dams					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-F0	- gestation (GD 8-	birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)			
Species: Chemical: HERO ID:	Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697475						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are pro- vided, and doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.			
Domain 6: Outcome Me	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Details of outcome assessment were not reported, only that the health of the dams was observed daily. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	Medium	Clinical signs are reported as negative in text.			
Additional Comments:	None						
Overall Quality Determination Medium							

Study Citation: Health Outcome(s) and Reported	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain						
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating					
Species: Chemical: HERO ID:	Rat-Sprague- Diethylhexyl 697475	Dawley - [rat]-Female Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	d Performance Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Medium	Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).			
Domain 3: Confounding	g / Variable Cor	trol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the pres- ence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.			
Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.			
Domain 5: Exposure Mo	ethods Sensitivi	ty					
Continued on next page							

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HERO ID: 697475 Table: 5 of 8

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Study Citation:	Gray, L., Ba	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.					
Health Outcome(s)	Mortality-N	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain					
and Reported							
Health Effect(s):	0.1.0						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-1-F0) - gestation (C	3D 8- birth)-F0- lactation (to PND 17)-F1- premating			
Exposure Route:	Dat Spragu	a Dawlay [rat] Famala					
Chemical.	Diethylhexy	/l Phthalate- Parent compound					
HERO ID:	697475	Trininade Turent compound					
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.			
Domain 6: Outcome Me	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm standard error. Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						
Overall Qualit	ty Deter	mination	High				

Study Citation: Health Outcome(s)	Gray, L., Bar rat: Added va Mortality-Mo	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain					
and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	Oral-Gavage Rat-Sprague Diethylhexyl	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating Rat-Sprague-Dawley - [rat]-Female					
HERO ID:	697475						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection and	d Performance						
	Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Medium	Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).			
Domain 3: Confounding	y / Variable Cor	atrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the pres- ence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.			
Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.			
Domain 5: Exposure M	ethods Sensitivi	ity					
	Continued on next page						

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Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 697475 Table: 6 of 8

		cont	tinued from p	revious page		
Study Citation:	Gray, L., Barat: Added	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.				
Health Outcome(s)	Mortality-N	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain				
and Reported						
Health Effect(s):						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-1-F0) - gestation (O	GD 8- birth)-F0- lactation (to PND 17)-F1- premating		
Exposure Route:						
Species:	Rat-Sprague	e-Dawley - [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	697475					
Domain		Metric	Rating	Comments		
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.		
Domain 6: Outcome Me	easures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.		
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means \pm standard error. Statistical analysis methods were reported and appropriate.		
Additional Comments:	None					
Overall Qualit	ty Deter	mination	High			

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides. Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697475				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Qu	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CASRN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Animals were pregnant on purchase but parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance Metric 2:	Allocation	High	Pregnant dams were randomly allocated to study groups based on body weight. Male pups were randomly selected from litter for the PUB cohort; the method was not re-	
	Metric 3:	Observational Bias / Blinding Changes	High	Assessors were blinded to treatment groups when assessing AGD and determining are- ola/nipple numbers and location. The study does not indicate if other endpoints were assessed blindly, although most were non-subjective (body and organ weights) or initial histopathological examination.	
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with stirring bar removed to avoid contact with plastics.	
Domain 4: Selective Rep	porting and At Metric 5:	trition Selective Reporting and Attrition	High	Data were reported for most outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block (two blocks included). Results are shown for 13-14 dams/group. The number of male offspring examined is reported in the results tables. There is no indication that any animals were excluded from the analysis.	
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		conti	inued from previ	ous page		
Study Citation:	Gray, L., Ba rat: Added y	rlow, N., Howdeshell, K., Ostby, J., Furr, J.	, Gray, C. (2009). ter Toxicological	Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) Sciences 110(2):411-425		
Health Outcome(s) and Reported Health Effect(s):	rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425. Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides.					
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-F0	- gestation (GD 8	- birth)-F0- lactation (to PND 17)-F1- premating		
Exposure Route:	Dat Care and					
Species: Chemical:	Diethylhexy	2-Dawley - [rat]-remaie				
HERO ID:	697475					
Domain		Metric	Rating	Comments		
Domain 5: Exposure N	lethods Sensitiv	vity				
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest and were justified by the study authors.		
Domain 6: Outcome M	easures and Re	sults Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. Based on the power analysis conducted by the authors, the number of males analyzed in this cohort was insufficient and lacked the statistical power to adequately detect reproductive histopathological changes. Doses chosen were similar to those previously reported findings in the literature. The methods of endpoint assessments were adequately described, appropriate, and sensitive to the outcomes of interest. Outcomes were assessed consistently across study groups. The test species and strain were appropriate and justified by the study authors.		
	Metric 9:	Results presentation	Low	Results were described in the text and data were presented in tables as means \pm standard error. Statistical analysis methods were reported and were appropriate in some cases; the litter was used as the statistical unit. Histopathology and gross malformation data for the PUB cohort alone were not adequately reported. Histopathology data were inappropriately combined and analyzed (across study cohorts and blocks, from two exposure durations), which significantly impacts the ability to interpret the study results. Individual animal data were not provided.		
Additional Comments:	None					
Overall Ouali	ty Deteri	nination	Medium			

Health Outcome(s) Other (please specify below) (Clinical signs)-Observational health of dams Including of please specify below) (Clinical signs)-Observational health of dams Including of please specify below) (Clinical signs)-Observational health of dams Including of please specify below) (Clinical signs)-Observational health of dams Including of please specify below) (Clinical signs)-Observational health of dams Including of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) (Clinical signs)-Observational health of dams Particle of please specify below) Particle of please specify below) Particle of please specify below) Particle of please specify below) Particle of please specified of please splease splease specified of please specified of please splease spl	Study Citation:	Gray, L., Bar	low, N., Howdeshell, K., Ostby, J., Furr, J., C	Gray, C. (2009).	Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD)		
Duration and Exposure Route: Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating Exposure Route: Species: Rat-Sprague-Dawley - [rat]-Female Chemical: Diethylhexyl Phthalate- Parent compound HERO ID: Gorments Domain Metric Rating Comments Domain 1: Reporting Quality Metric Reporting Quality Medium Metric 1: Reporting Quality Medium The chemical was identified by name (di(2-ethylhexyl)phthalate (DEIP)) with CAS EN 117-81-7 CASIN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test atimal appecies, strin, as, e.g., and source were reported. Domain 2: Selection and Performance Metric 2: Allocation Metric 3: Observational Bias / Blinding Changes High Medium Animals were randomly allocated to study groups based on body weights. Domain 3: Confounding / Variable Control Metric 4: Confounding / Variable Control Medium An ergative control group is not necessary for this type of study. Housing conditions that were specified seeme of consistent across group. Driving vas analyzed for the yres and a stude of chemicals (e.g. periodic and hear what has the removed to avoid contact with glassist. This lack of details is not expected to have a significant import on the endpoints described. Domain 3: Confounding / Variable Control Metric 4: Confounding / Variable Control Medium <t< th=""><th>Health Outcome(s) and Reported Health Effect(s):</th><th colspan="6" rowspan="2">Other (please specify below) (Clinical signs)-Observational health of dams Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating</th></t<>	Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observational health of dams Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating					
Exposure Route: Species: Rat-Sprague-Dawley - [rat]-Female Chemical: Dicthylbexyl Phthalate-Parent compound 697475 Domain Metric Rating Comments Domain 1: Reporting Quality Metric Redium The chemical was identified by name (di(2-ethylbexyl)phthalate (DEIPI)) with CAS RN 117-81-7 CASRN and lot number: The source and purity (99.15) of the test substance were reported. Test animal species. Strain, sex, age, and source were reported. Domain 2: Selection and Performance Metric 2: Allocation High Metric 3: Observational Bias / Blinding Changes High Animals were randomly allocated to study groups based on body weights. Domain 3: Confounding / Variable Control Medium An engative control group was included and responses were appropriate. A positive control group. This type of study. Housing conditions that were specified segment to indicate to study groups. Subset of no bad water were specified segment to indicate to be consistent across group. Drinking ware was allocated to be consistent across group. Drinking ware was allocated for clinical signs. Domain 3: Confounding / Variable Control Medium A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified segment to a location of whether tost animal species, strain across group. Drinking water was allocated for paedomonas and a stude of chemicals caps were toported. The study does report that doing	Duration and						
Species: Rat-Sprague-Jawkey - [rai]-female Chemical: Dietylhexyl Phthalate- Parent compound HERO ID: 697475 Domain 1: Metric Rating Comments Domain 1: Reporting Quality Medium The chemical was identified by name (dir.2-ehylhexyl)phthalate [DEHP]) with CAS Party was not reported. Test namesce and purity OPIs) of the test substance were reported. Test namal species, strain, sex, age, and source were reported. Domain 2: Selection and Performance Metric 3: Observational Bias / Blinding Changes Metric 3: Observational Bias / Blinding Changes Heigh Animals were reported. To study groups based on body weights. Domain 3: Confounding / Variable Control Medium A negative control group was included and responses were appropriate. A positive control group was included and responses were used heady of the response of study. Housing conditions that were specified seemed to be consistent across groups. Disinking conditions that were specified seemed to be consistent across groups. Disinking conditions that were specified and vater disensing contains were specified to avoid company as included and responses were appropriate. A positive control group was included and responses were used ind	Exposure Route:	D . 0					
Domain Metric Rating Comments Domain 1: Reporting Quality Metric 1: Reporting Quality The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and to number. The source and purity (99.1%) of the test stud-stance were reported. Thest animal species, strain, sex, age, and source were reported. The trainable constraines, humidity, get and source were reported. The traines of animals/cage was not reported. Lushadry conditions (remperture, humidity, fight ycc) were reported. Endpoint evaluation methods were reported. Source and purity (99.1%) of the test stud-stance were reported. The mumber of animals/cage was not reported. Cage type and bedding type were reported. Endpoint evaluation methods were reported. Source and were reported. Endpoint evaluation methods were reported. Source and were reported. Endpoint evaluation methods were reported. Source and purity (99.1%) of the test stud-stance were reported. Source and purity (99.1%) of the test stud-stance were reported. Source and purity was not reported. Initial body weights were not reported. Endpoint evaluation methods were reported. Initial body weights were not reported. Endpoint evaluation methods were reported. Source and purity (99.1%) of the test stud-stance and performance Metric 2: Allocation Metric 3: Observational Bias / Blinding Changes Domain 3: Confounding / Variable Control Medium Animals were randomly allocated to study groups based on body weights. The were was also no indication of whether est animal bedding of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and lested for marks on the data with the stir bar removed to avaid out on indication of whether est animal bedding of study. Housing conditions that wer	Species: Chemical: HERO ID:	Rat-Sprague Diethylhexyl 697475	-Dawley - [rat]-Female Phthalate- Parent compound				
Domain 1: Reporting Quality Metric 1: Reporting Quality Medium The chemical was identified by name (dit2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not perforted. Heading conditions was not reported. Cage type and bedding type were reported. Test animal species, strain, sex, age, and source were reported. Domain 2: Selection and Performance Metric 2: Allocation Metric 3: Observational Bias / Blinding Changes High Animals were randomly allocated to study groups based on body weights. Domain 3: Confounding / Variable Control Medium Metric 4: Confounding / Variable Control Metric 5: Selective Reporting and Attrition Domain 4: Selective Reporting and Attrition High Domain 5: Emosure Methods Sensitivity Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.	Domain		Metric	Rating	Comments		
Domain 2: Selection and Performance Metric 2: Allocation High Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias / Blinding Changes High Medium Blinding or other measures to reduce observational bias were not reported for clinical signs. Domain 3: Confounding / Variable Control Metric 4: Confounding / Variable Control Medium A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Dolycarbonate cages were used to be consistent across proups. Drolycarbonate cages were used to be consistent across the attrostice. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described. Domain 4: Selective Reporting and Attrition High Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group. Domain 5: Exposure Methods Sensitivity Domain 5: Exposure Methods Sensitivity	Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test sub- stance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and Pointmance Allocation Metric 2: Allocation Metric 3: Observational Bias / Blinding Changes Medium Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported for clinical signs. Domain 3: Confounding / Variable Control Metric 4: Confounding / Variable Control Medium A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Dirinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycenotate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to consta control thy lipatities. This lack of details is not expected to have a significant impact on the endpoints described. Domain 4: Selective Reporting and Attrition High Metric 5: Selective Reporting and Attrition Metric 5: Selective Reporting and Attrition Metric 5: Selective Reporting and Attrition Metric 5: Selective Reporting and Attrition <t< td=""><th>Domain 2: Selection an</th><td>d Performance</td><td></td><td></td><td></td></t<>	Domain 2: Selection an	d Performance					
Domain 3: Confounding / Variable Control Medium A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described. Domain 4: Selective Reporting and Attrition High Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group. Domain 5: Exposure Methods Sensitivity. Domain 5: Exposure Methods Sensitivity.		Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	High Medium	Animals were randomly allocated to study groups based on body weights. Blinding or other measures to reduce observational bias were not reported for clinical signs.		
Domain 5: Confounding / Variable Control Medium A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described. Domain 4: Selective Reporting and Attrition High Detat were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.	Domain 3: Confounding	o / Variable Cor	ntrol				
Domain 4: Selective Reporting and Attrition Metric 5: Selective Reporting and Attrition High Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group. Domain 5: Exposure Methods Sensitivity Domain 5: Exposure Methods Sensitivity		Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the pres- ence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.		
Domain 5: Exposure Methods Sensitivity	Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.		
Continued on next page	Domain 5: Exposure M	ethods Sensitiv	ity Contin	ued on next pa	ΩΦ		

May 2025 Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697475 Table: 8 of 8

		conti	nued from previ	ous page			
Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.						
Health Outcome(s) and Reported	Other (pleas	Other (please specify below) (Clinical signs)-Observational health of dams					
Health Effect(s):							
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-F0	- gestation (GD 8	- birth)-F0- lactation (to PND 17)-F1- premating			
Exposure Route:							
Species:	Rat-Sprague	e-Dawley - [rat]-Female					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	697475						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not inde- pendently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are pro vided, and doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.			
Domain 6: Outcome Me	asures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Details of outcome assessment were not reported, only that the health of the dams was observed daily. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	Medium	Clinical signs are reported as negative in text.			
Additional Comments:	None						

Study Citation:	Guo, J., Li, Z	X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q.	Q., Ge, R. S. (2	2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes			
Health Outcome(s) and Reported	from the diff Reproductive ber, testes his	Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell num- ber, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers					
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	-Duration: Short-term (>1-30 days)-11-day((s)-Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-day(s)			
Species:	Rat-Long-Ev	/ans - [rat]-Male					
Chemical:	Diethylhexy	Phthalate- Parent compound					
HERO ID:	2001148						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes be- ing assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.			
Domain 3: Confounding	a / Variable Cou	atrol					
	Metric 4:	Confounding / Variable Control	Low	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.			
Domain 4: Salaatiya Pa	norting and Att	rition					
Domain 4. Selective Re	Metric 5	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were provided for all prespecified outcomes, expo			
	Metric 5.		Wedium	sure groups, and time points. No animal attrition was reported (all animals survived the study). Not all animals are accounted for in the results, as most endpoints present a sample size of 4 or 5 or present a range of 4-6 (with a sample size of 6/group reported in the methods). These omissions are not explained but the impact on the results is likely to be minor.			
Domain 5: Exposure M	ethods Sensitiv	ity					
		Contin	ued on next pa				

Diethylhexyl Phthalate

		conti	nued from previ	ous page	
Study Citation:	Guo, J., Li, from the dif	X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q ferentiation of stem cells into Levdig cell li	Q. Q., Ge, R. S. (2 nease in the adult	013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes	
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell num- ber, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers				
Duration and Exposure Route:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-11-da	y(s)-Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-day(s)	
Species:	Rat-Long-E	vans - [rat]-Male			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	2001148				
Domain		Metric	Rating	Comments	
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.	
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate consider- ing the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.	
Domain 6: Outcome M	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the out- comes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent, and outcome assessment methodologies were described in detail and appropriately ad- dressed the intended outcomes. Testes were fixed in Bouin's solution, which may lead to differential tubular shrinkage. This is not expected to substantially impact results.	
	Metric 9:	Results presentation	High	Results are presented for all outcomes using bar graphs that provide adequate detail for interpretation of results, including indicators for statistical significance and error bars. Results are available for all exposure groups and statistical methods were adequately described and appropriate.	
Additional Comments:	None				

Overall Quality Determination

Medium

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Guo, J., Li, 2 from the difference Reproductive ber, testes his Oral-Gavage Rat-Long-Ev Diethylhexyl 2001148	K. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. erentiation of stem cells into Leydig cell line //Developmental-Serum testosterone and lut stpathology, Leydig cell stage, mRNA conce -Duration: Short-term (>1-30 days)-11-day ans - [rat]-Male Phthalate- Parent compound	Q., Ge, R. S. (2 eage in the adult einizing hormor entrations of Ley (s)-Oral-Gavage	2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes t rat testis. Toxicology 306:9-15. ne concentrations, steroidogenic enzyme concentration/activity, Leydig cell num- ydig cell specific markers e-Duration: Short-term (>1-30 days)-7-day(s)
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and	1 Performance			
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes be- ing assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.
Domain 3: Confounding	y / Variable Cor	itrol		
	Metric 4:	Confounding / Variable Control	Low	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.
Domain 4: Selective Re	porting and Att	rition		
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were provided for all prespecified outcomes, expo- sure groups, and time points. No animal attrition was reported (all animals survived the study). Not all animals are accounted for in the results, as most endpoints present a sam- ple size of 4 or 5 or present a range of 4-6 (with a sample size of 6/group reported in the methods). These omissions are not explained but the impact on the results is likely to be minor.
Domain 5: Exposure Me	ethods Sensitivi	ity		
		Contin	ued on next pa	ge

Diethylhexyl Phthalate

		conti	nued from previ	ous page			
Study Citation:	Guo, J., Li, from the dif	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15. Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell num-					
Health Outcome(s)	Reproductiv						
and Reported	ber, testes h	ber, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers					
Health Effect(s):							
Duration and	Oral-Gavag	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)					
Exposure Route:							
Species:	Rat-Long-E	vans - [rat]-Male					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	2001148						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate consider- ing the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.			
Domain 6: Outcome M	easures and Re	esulte Dienlay					
	Metric 9:	Endpoint sensitivity and specificity Results presentation	Medium High	Dose levels were selected based on results of previous studies that investigated the out- comes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent, and outcome assessment methodologies were described in detail and appropriately ad- dressed the intended outcomes. Testes were fixed in Bouin's solution, which may lead to differential tubular shrinkage. This is not expected to substantially impact results. Results are presented for all outcomes using bar graphs that provide adequate detail for			
			<i>.</i>	interpretation of results, including indicators for statistical significance and error bars. Results are available for all exposure groups and statistical methods were adequately described and appropriate.			

Additional Comments: None

Overall Quality Determination

Medium

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Study Citation: Health Outcome(s) and Reported	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15. Mortality-Death-Nutritional/Metabolic-Body weights and food consumption					
Duration and Exposure Route:	Oral-Gavag	e-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-day(s)		
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 2001148	vans - [rat]-Male /l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.		
Domain 2: Selection an	d Performance					
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes be- ing assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.		
Domain 3: Confoundin	r / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information is not likely to significantly impact these endpoints.		
Domain 4: Salaatiya Da	norting and A	ttrition				
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for these outcomes (no mortalities and no effects on body weights or food consumption). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.		
Domain 5: Exposure M	ethods Sensitiv	vity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.		
		Contin	ued on next pa	nge		

		conti	inued from previ	ous page	
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Guo, J., Li, Z from the diff Mortality-Do	X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q ferentiation of stem cells into Leydig cell li eath-Nutritional/Metabolic-Body weights a	Q. Q., Ge, R. S. (2 ineage in the adult and food consump	013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes rat testis. Toxicology 306:9-15. tion	
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)				
Species:	Rat-Long-E	vans - [rat]-Male			
Chemical: HERO ID:	Diethylhexyl 2001148	l Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate consider- ing the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.	
Domain 6: Outcome Mea	asures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the out- comes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodology was not reported, but missing information is not likely to influence the results.	
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were ob- served. Statistical analyses were appropriate, but data were not provided to confirm statistics.	
Additional Comments:	None				

Study Citation: Health Outcome(s) and Reported Health Effect(s):	Guo, J., Li, from the dif Mortality-D	X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. ferentiation of stem cells into Leydig cell line eath-Nutritional/Metabolic-Body weights and	Q., Ge, R. S. (2 eage in the adult l food consump	2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes a rat testis. Toxicology 306:9-15. tion
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-11-day	(s)-Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-day(s)
Exposure Route: Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 2001148	vans - [rat]-Male vl Phthalate- Parent compound		
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and	d Performance			
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes be- ing assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.
Domain 3: Confounding	y / Variable Co	ontrol		
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information is not likely to significantly impact these endpoints.
Domain 4: Selective Re	norting and At	ttrition		
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for these outcomes (no mortalities and no effects on body weights or food consumption). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.
Domain 5: Exposure M	ethods Sensitiv	vity		
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes					
Health Outcome(s)	from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.					
and Reported	Wortanty-D	eath-Nutritional/Metabolic-body weights a	ina 100a consump			
Health Effect(s):						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-11-da	v(s)-Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-day(s)		
Exposure Route:	8		.)(s) since since ge			
Species:	Rat-Long-E	vans - [rat]-Male				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	2001148					
Domain		Metric	Rating	Comments		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate consider- ing the purpose of the study to investigate effects of adult exposure on number of Leydi- cells in the testis.		
Domain 6: Outcome M	easures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the out- comes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent.		
				The outcome assessment methodology was not reported, but missing information is not likely to influence the results.		
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were ob- served. Statistical analyses were appropriate, but data were not provided to confirm statistics.		
Additional Commonta	None					

Study Citation: Health Outcome(s) and Reported	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15. Other (please specify below) (Clinical signs)-Activity of animals						
Health Effect(s): Duration and Exposure Route:	Oral-Gavag	e-Duration: Short-term (>1-30 days)-11-day	(s)-Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-day(s)			
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 2001148	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 2001148					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.			
	Metric 3:	Observational Bias / Blinding Changes	Low	Methods for reducing observational bias were not reported, and the outcome being as- sessed is subjective in nature. Therefore, a lack of blinding for this outcome may have impacted study results.			
Domain 3: Confounding	y / Variable Co	ontrol					
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.			
Domain 1: Selective Re	porting and At	ttrition					
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for this outcome (no effect on animals' activity). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.			
Domain 5: Exposure M	athode Sansitiv						
	Metric 6:	Chemical administration and characterization	Low	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.			

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Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15. Other (please specify below) (Clinical signs)-Activity of animals				
Exposure Route:	Rat-Long-F	vans - [rat] Male	iy(s)-Orai-Gavage		
Chemical: HERO ID:	Diethylhexy 2001148	l Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate consider- ing the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.	
Domain 6: Outcome M	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	Low	Dose levels were selected based on results of previous studies that investigated the out- comes of interest. Test animals were obtained from a commercial source and the species strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodol- ogy was not reported, and the outcome of interest was not described in detail, so it was unclear whether methods were sensitive for the outcome of interest or what the authors intended to evaluate for clinical signs.	
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were ob- served. Statistical analyses were appropriate, but data were not provided to confirm statistics.	
Additional Comments:	None				

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Study Citation: Health Outcome(s) and Reported	Guo, J., Li, from the dif Other (pleas	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15. Other (please specify below) (Clinical signs)-Activity of animals					
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s) Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 2001148						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.			
	Metric 3:	Observational Bias / Blinding Changes	Low	Methods for reducing observational bias were not reported, and the outcome being as- sessed is subjective in nature. Therefore, a lack of blinding for this outcome may have impacted study results.			
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food			
				consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproduc- tive/developmental endpoints.			
Damain 4. Salastina Da							
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for this outcome (no effect on animals' activity). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.			
Domain 5: Exposure M	ethods Sonsiti	vity					
	Metric 6:	Chemical administration and characterization	Low	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.			
		Contin	ued on next pa	nge			

Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15. Other (please specify below) (Clinical signs)-Activity of animals				
Exposure Route:	Rat-Long-F	vans - [rat] Male	iy(s)-Orai-Gavage		
Chemical: HERO ID:	Diethylhexy 2001148	l Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate consider- ing the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.	
Domain 6: Outcome M	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	Low	Dose levels were selected based on results of previous studies that investigated the out- comes of interest. Test animals were obtained from a commercial source and the species strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodol- ogy was not reported, and the outcome of interest was not described in detail, so it was unclear whether methods were sensitive for the outcome of interest or what the authors intended to evaluate for clinical signs.	
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were ob- served. Statistical analyses were appropriate, but data were not provided to confirm statistics.	
Additional Comments:	None				

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Study Citation:	Kitaoka, M.,	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the				
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Food consumption, water consumption, and body weight					
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Du (>30-90 day Mouse-A/J - Diethylhexyl 2000828	uration: Short-term (>1-30 days)-7-2-week s)-7-8-week(s) [mouse]-Male l Phthalate- Parent compound	(s)-Oral-Diet-D	Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic		
Domain		Metric	Rating	Comments		
Domain 1: Reporting Qu	ality Metric 1:	Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.		
Domain 2: Selection and	l Performance					
	Metric 2:	Allocation	Medium	Study authors state that mice were randomly allocated into study groups, method used was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the interpretation of the results.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (body weight, and food and water intake).		
Domain 3: Confounding	/ Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results. Polycar- bonate cages were used instead of wire cages. Food and water dispensing containers were not described.		
Domain 4: Selective Rep	porting and Att	rition				
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Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported	Kitaoka, M. local immur Nutritional/	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Nutritional/Metabolic-Food consumption, water consumption, and body weight					
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 2000828						
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Low	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition.Data for body weight and food intake were not appropriately reported. Not all timepoints were reported independently and it cannot be determined which timepoint the data presented pertains to.			
Domain 5: Exposure M	lethods Sensitiv	ity.					
Domain 5: Exposure w	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and duration	Low High	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results. For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.			
Domain 6: Outcome M	easures and Re Metric 8:	sults Display Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups $(0, 0.01\%, 0.1\% \text{ DEHP})$ was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.			
	Metric 9:	Results presentation	Uninformative	Body weight data was reported however it is unclear which time point these data pertain to. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state ter- minal body weights were recorded for each. Table 1 reports body weight data but does not indicate which timepoint this is for. Lack of this information makes this endpoint uninformative. Mean food intake was reported however which timepoint these values pertain to or how many animals were included in the calculation were not reported. Wa- ter intake is reported as an approximation of 7 ml/day.			
Additional Comments:	None						

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 2000828 Table: 1 of 4

Diethylhexyl Phthalate

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Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, M local immunity in the testis by exposure to di	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490.					
Health Outcome(s)	Nutritional/Metabolic-Food consumption, wa	ter consumption, and body weight	-				
and Reported							
Health Effect(s):							
Duration and	Oral-Diet-Duration: Short-term (>1-30 day	s)-7-2-week(s)-Oral-Diet-Duration: Short	term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic				
Exposure Route:	(>30-90 days)-7-8-week(s)						
Species:	Mouse-A/J - [mouse]-Male	Mouse-A/J - [mouse]-Male					
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	2000828						
Domain	Metric	Rating	Comments				
Overall Qual	ity Determination	Medium					

Study Citation: Health Outcome(s) and Reported Health Effect(s):	Kitaoka, M. local immu Nutritional/	taoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the cal immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. ttritional/Metabolic-Food consumption, water consumption, and body weight				
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 2000828					
Domain		Metric	Rating	Comments		
Domain 1: Reporting (Quality Metric 1:	Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.		
Domain 2: Selection and	nd Performance Metric 2:	Allocation	Medium	Study authors state that mice were randomly allocated into study groups, method used was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the inter-		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (body weight, and food and water intake).		
Domain 2: Confoundir	va / Variabla Ca	ntrol				
Joniani J. Comoundii	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results. Polycar- bonate cages were used instead of wire cages. Food and water dispensing containers were not described.		
Domain 4: Selective R	eporting and A	trition				
		Contin	ued on next pa	nge		

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Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Nutritional/Metabolic-Food consumption, water consumption, and body weight					
Exposure Route: Species: Chemical:	(>30-90 day Mouse-A/J - Diethylhexy	//s)-7-8-week(s) · [mouse]-Male l Phthalate- Parent compound		ration: Short-term (>1-50 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic		
HEROID:	2000828	M-t-:-	Datina	Comments		
Domain	Metric 5:	Selective Reporting and Attrition	Low	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition.Data for body weight and food intake were not appropriately reported. Not all timepoints were reported independently and it cannot be determined which timepoint the data presented pertains to.		
Domain 5: Exposure M	ethods Sensitiv	rity				
Domain 5. DAposare M	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.		
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.		
Domain 6: Outcome M	easures and Re	sults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.		
	Metric 9:	Results presentation	Uninformative	Body weight data was reported however it is unclear which time point these data pertain to. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state ter- minal body weights were recorded for each. Table 1 reports body weight data but does not indicate which timepoint this is for. Lack of this information makes this endpoint uninformative. Mean food intake was reported however which timepoint these values pertain to or how many animals were included in the calculation were not reported. Wa- ter intake is reported as an approximation of 7 ml/day.		

Additional Comments: None

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Human Health Hazard Animal Toxicology Evaluation

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Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, N	M., Qu, N., Hatayama, N., Miyaso, H., Mat	suno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the		
Health Outcome(s)	Nutritional/Metabolic-Food consumption, wa	ter consumption, and body weight	Journal of Reproduction and Development 59(5):485-490.		
and Reported Health Effect(s):					
Duration and	Oral-Diet-Duration: Short-term (>1-30 day	s)-7-2-week(s)-Oral-Diet-Duration: Short-	term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic		
Exposure Route:	(>30-90 days)-7-8-week(s)				
Species:	Mouse-A/J - [mouse]-Male				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	2000828				
Domain	Metric	Rating	Comments		
Overall Quali	ty Determination	Medium			

Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490.
Health Outcome(s)	Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of
and Reported	seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase
Health Effect(s):	detection)
Duration and	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic
Exposure Route:	(>30-90 days)-7-8-week(s)
Species:	Mouse-A/J - [mouse]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000828

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.
Domain 2: Selection and Performance			
Metric 2:	Allocation	Medium	Study authors state that mice were randomly allocated into study groups, method used was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the interpretation of the results.
Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Co	ntrol		
Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and valid- ity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 2000828 Table: 3 of 4

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continued from previous page						
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase detection)					
Duration and Exposure Poute:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic					
Species:	(>30-90 days)-7-8-week(s) Mouse-A/I - [mouse]-Male					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	2000828					
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantita- tive or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.		
Domain 5: Exposure Methods Sensitivity						
Domani 5. Exposure Mi	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.		
	Metric 7:	Exposure timing, frequency, and	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest		
		uuration		study type and endpoints of interest.		
Domain 6: Outcome Measures and Results Display						
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.		
	Metric 9:	Results presentation	Medium	Data for testis weight is not appropriately reported. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state testes weights was recorded for each timepoint. Table 1 reports testes weight data but does not indicate which timepoint this is for. Histopathology of testes was reported sufficiently with means and SD. Statistical analysis was performed and appropriate. Although testicular weight data cannot be used for this assessment, the histopathological data is adequately reported.		

Additional Comments: None

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Health Outcome(s) Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase and Reported **Health Effect(s):** detection) **Duration and** Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic **Exposure Route:** (>30-90 days)-7-8-week(s) Species: Mouse-A/J - [mouse]-Male **Chemical:** Diethylhexyl Phthalate- Parent compound **HERO ID:** 2000828 Domain Metric Rating Comments

Overall Quality Determination

Medium
Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490.
Health Outcome(s)	Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of
and Reported	seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase
Health Effect(s):	detection)
Duration and	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic
Exposure Route:	(>30-90 days)-7-8-week(s)
Species:	Mouse-A/J - [mouse]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000828

Metric	Rating	Comments
Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.
e		
Allocation	Medium	Study authors state that mice were randomly allocated into study groups, method used was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the interpretation of the results.
Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
ontrol		
Confounding / Variable Control	Low	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and valid- ity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described
	e Allocation Observational Bias / Blinding Changes ontrol Confounding / Variable Control	Metic Kaing Reporting Quality Medium e Allocation Medium Observational Bias / Blinding Changes Medium ontrol Confounding / Variable Control Low

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PUBLIC RELEASE DRAFT May 2025

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 2000828 Table: 4 of 4

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		conti	inued from previ	ous page		
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase detection) Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 					
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantita- tive or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.		
Domain 5: Exposure M	ethods Sensitiv	ity				
Domani J. Exposure M	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.		
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.		
Domain 6: Outcome M	ansuras and Da	culte Dieploy				
Domain 0. Outcome Me	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.		
	Metric 9:	Results presentation	Medium	Data for testis weight is not appropriately reported. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state testes weights was recorded for each timepoint. Table 1 reports testes weight data but does not indicate which timepoint this is for. Histopathology of testes was reported sufficiently with means and SD. Statistical analysis was performed and appropriate. Although testicular weight data cannot be used for this assessment, the histopathological data is adequately reported.		

Additional Comments: None

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Health Outcome(s) Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase and Reported **Health Effect(s):** detection) **Duration and** Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic **Exposure Route:** (>30-90 days)-7-8-week(s) Species: Mouse-A/J - [mouse]-Male **Chemical:** Diethylhexyl Phthalate- Parent compound **HERO ID:** 2000828 Domain Metric Rating Comments

Overall Quality Determination

Medium

Study Citation:	Kwack, S., I	Kim, K., Kim, H., Lee, B. (2009). Comparat	tive toxicolo	gical evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for				
Health Outcome(s) and Reported Health Effect(s):	risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Mortality-Mortality							
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-4-w	eek(s)					
Species:	Rat-Sprague	e-Dawley - [rat]-Male						
Chemical:	Diethylhexy	l Phthalate- Parent compound						
HERO ID:	697382							
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	Quality Metric 1:	Reporting Quality	Medium	All of the critical information was reported, including test animal species, test substance (name, CAS. No., molecular weight, chemical structure), dose and duration of expo- sure, route, and results for at least one endpoint. Most of the important information was also reported. The test animal source, strain, age, sex, and starting body weights were reported, along with the general husbandry conditions (temperature, humidity, venti- lation, light- dark cycle, diet, water availability), although the number of animals per cage was not reported. The test animal was obtained from a commercial source and were an appropriate animal model for the study. A list of sources for the test substances was provided, although it is unclear which substances came from which sources. The pu- rity/grade were not reported. The frequency of exposure (assumed 1/day, 7 days/week) and number of animals per exposure group (figures show 5-6 animals) were not explicitly described. The assays used to evaluate the outcomes were adequately reported.				
Domain 2: Selection an	nd Performance	Allocation	Madium					
	Wieure 2:	Anocation	Mediulii	specific methods were not described.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes was a simple objective measure.				
Domain 3: Confoundin	g / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	Not enough information was reported to determine confounding. A negative control group was used and similarly gavaged with corn oil alone. A positive control is not required for this type of study. Food consumption was measured and similar across control and treated animals (negative results reported qualitatively). Water intake was not reported. There is no indication that there were differences in husbandry conditions between the control and treatment groups.				
Domain 4: Selective Re	eporting and At	trition						
		Contin	nued on nex	t page				

		con	tinued from p	previous page				
Study Citation: Health Outcome(s)	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Mortality-Mortality							
and Reported								
Health Effect(s):	0.10		1 ()					
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-1-4-	-week(s)					
Exposure Koute:	Dot Spragu	Dowlay [rat] Mala						
Species: Chemical:	Diethylbey	d Phthalate- Parent compound						
HERO ID:	697382	T i initialate- T arent compound						
Domain	077002	Metric	Rating	Comments				
Domain	Metric 5:	Selective Reporting and Attrition	Low	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure. Urinalysis results were not reported quantitatively, although an incomplete qualitative statement was present (some parameters were changed in some groups, but no indication of the direction or magnitude of the changes). The number of animals per group was not defined in the methods, although most tables showed n=6. However, the body weight graph stated that the data represented 5 to 6 animals, and it is not clear why some of the animals were missing or which groups had 5 or 6 animals.				
Domain 5: Exposure M	lethods Sensitiv Metric 6:	vity Chemical administration and characterization	Low	The test substance was identified definitively (name, CAS No., structure). A list of sources was provided, although it is unclear which substance came from which source. The purity and/or grade of test substance were not reported, and there is no indication that the purity was tested. No information was reported on the preparation or storage of the test substance. The dose was reported, but no mention of analytical verification. The route and method of exposure were reported and appropriate for the test substance, but the test volume was not reported.				
	Metric 7:	Exposure timing, frequency, and duration	Low	Details of the exposure administration were incompletely reported. There is no infor- mation on the timing of the dosing, and the frequency of dosing is not explicitly stated (assuming 1x/day, 7 days/week). There is not enough information to determine if the exposures were administered consistently between treatment groups.				
Domain 6: Outcome M	easures and Re	sults Display						
	Metric 8:	Endpoint sensitivity and specificity	Medium	Only a single dose was tested, and the concentration selection was not justified. The test animal was obtained from a commercial source and were appropriate for the study. The animal numbers per group were not reported, although most tables suggested that there were 5-6 males in each group. The outcome assessment protocols were reported, although there is not enough information to determine if they were evaluated consistently, such as time of day. The outcome methodology addressed the intended outcome.				
	Metric 9:	Results presentation	Medium	Data were presented qualitatively (no animals died), and statistical analysis not required.				
Additional Comments:	None							
Overall Quali	ty Deteri	nination	Low					

Study Citation: Health Outcome(s) and Reported	Kwack, S., I risk assessm Nutritional/I	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Nutritional/Metabolic-Body weight, food consumption					
Health Effect(s): Duration and Exposure Poute:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-1-4-w	eek(s)				
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 697382	e-Dawley - [rat]-Male l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All of the critical information was reported, including test animal species, test substance (name, CAS. No., molecular weight, chemical structure), dose and duration of expo- sure, route, and results for at least one endpoint. Most of the important information was also reported. The test animal source, strain, age, sex, and starting body weights were reported, along with the general husbandry conditions (temperature, humidity, venti- lation, light- dark cycle, diet, water availability), although the number of animals per cage was not reported. The test animal was obtained from a commercial source and were an appropriate animal model for the study. A list of sources for the test substances was provided, although it is unclear which substances came from which sources. The purity/grade were not reported. The frequency of exposure (assumed 1/day, 7 days/week) and number of animals per exposure group (figures show 5-6 animals) were not explicitly described. The assays used to evaluate the outcomes were adequately reported.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Medium	The animals were randomly allocated to groups based on their body weight, but the specific methods were not described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes was a simple objective measure.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	Not enough information was reported to determine confounding. A negative control group was used and similarly gavaged with corn oil alone. A positive control is not required for this type of study. Food consumption was measured and similar across control and treated animals (negative results reported qualitatively). Water intake was not reported. There is no indication that there were differences in husbandry conditions between the control and treatment groups.			
Domain 4: Selective Reporting and Attrition							
	Metric 5:	Selective Reporting and Attrition	Low	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure. Urinalysis results were not reported quantitatively, although an incomplete qualitative statement was present (some parameters were changed in some groups, but no indication of the direction or magnitude of the changes). The number of animals per group was not defined in the methods, although most tables showed n=6. However, the body weight graph stated that the data represented 5 to 6 animals, and it is not clear why some of the animals were missing or which groups had 5 or 6 animals.			

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Diethylhexyl Phthalate

HERO ID: 697382 Table: 2 of 5

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Study Citation:	Kwack, S.,	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health. Part A: Current Issues 72(21-22):1446-1454					
Health Outcome(s) and Reported Health Effect(s):	 Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s) 						
Duration and Exposure Route:							
Species: Chemical: HERO ID:	Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound 697382						
Domain		Metric	Rating	Comments			
Domain 5: Exposure M	ethods Sensitiv	vitv					
2 onian of 2.1900are 11	Metric 6:	Chemical administration and characterization	Low	The test substance was identified definitively (name, CAS No., structure). A list of sources was provided, although it is unclear which substance came from which source. The purity and/or grade of test substance were not reported, and there is no indication that the purity was tested. No information was reported on the preparation or storage of the test substance. The dose was reported, but no mention of analytical verification. The route and method of exposure were reported and appropriate for the test substance, but the test volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	Low	Details of the exposure administration were incompletely reported. There is no infor- mation on the timing of the dosing, and the frequency of dosing is not explicitly stated (assuming 1x/day, 7 days/week). There is not enough information to determine if the exposures were administered consistently between treatment groups.			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	Only a single dose was tested, and the concentration selection was not justified. The test animal was obtained from a commercial source and were appropriate for the study. The animal numbers per group were not reported, although most tables suggested that there were 5-6 males in each group. The outcome assessment protocols were reported, although there is not enough information to determine if they were evaluated consistently. The outcome methodology addressed the intended outcome.			
	Metric 9:	Results presentation	Medium	Data were presented graphically with the appropriate statistical analysis, although it was difficult to determine the quantitative results.			
Additional Comments:	None						
Overall Quali	ty Deteri	mination	Low				

Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health. Part A: Current Journal of 72(21-22):1446-1454						
Health Outcome(s) and Reported Health Effect(s):	risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues /2(21-22):1446-1454. Other (please specify below) (Clinical signs, endocrine)-Clinical signs, adrenal gland weight-Hepatic/Liver-Liver weight, serum chemistry (choles- terol, triglyceride, total bilirubin, total protein, alkaline phosphatase, glutamate pyruvate, glutamate oxaloacetate transaminase, g-gluamyl transferase)- Renal/Kidney-Kidney weight, serum chemistry (calcium, potassium, sodium, albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood, pH, protein, urobilinogen, glucose, nitrite, bilirubin, ketone bodies, leukocytes, urine specific gravity)-Immune/Hematological-Spleen and thymus weights, hematology (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count)-Lung/Respiratory-Lung weight-Cancer/Carcinogenesis-Heart weight-Thyroid-Thyroid weight						
Exposure Route:	Ofui Ouvug	Duration. Short term (>1 50 days) 1 1 w	ccr(3)				
Species:	Rat-Sprague	e-Dawley - [rat]-Male					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	697382						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All of the critical information was reported, including test animal species, test substance (name, CAS. No., molecular weight, chemical structure), dose and duration of expo- sure, route, and results for at least one endpoint. Most of the important information was also reported. The test animal source, strain, age, sex, and starting body weights were reported, along with the general husbandry conditions (temperature, humidity, venti- lation, light- dark cycle, diet, water availability), although the number of animals per cage was not reported. The test animal was obtained from a commercial source and were an appropriate animal model for the study. A list of sources for the test substances was provided, although it is unclear which substances came from which sources. The purity/grade were not reported. The frequency of exposure (assumed 1/day, 7 days/week) and number of animals per exposure group (figures show 5-6 animals) were not explicitly described. The assays used to evaluate the outcomes were adequately reported.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Medium	The animals were randomly allocated to groups based on their body weight, but the specific methods were not described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes were based on use of automated/computer-driven systems, standard laboratory kits, or simple objective measures.			
Domain 3: Confounding	Domain 3: Confounding / Variable Control						
	Metric 4:	Confounding / Variable Control	Medium	Not enough information was reported to determine confounding. A negative control group was used and similarly gavaged with corn oil alone. A positive control is not required for this type of study. Food consumption was measured and similar across control and treated animals (negative results reported qualitatively). Water intake was not reported. There is no indication that there were differences in husbandry conditions between the control and treatment groups.			
Domain 4: Selective Reporting and Attrition							
Continued on next page							

PUBLIC RELEASE DRAFT May 2025

Human Health Hazard Animal Toxicology Evaluation

		conti	inued from p	previous page			
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Other (please specify below) (Clinical signs, endocrine)-Clinical signs, adrenal gland weight-Hepatic/Liver-Liver weight, serum chemistry (cholesterol, triglyceride, total bilirubin, total protein, alkaline phosphatase, glutamate pyruvate, glutamate oxaloacetate transaminase, g-gluamyl transferase)-Renal/Kidney-Kidney weight, serum chemistry (calcium, potassium, sodium, albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood, pH, protein, urobilinogen, glucose, nitrite, bilirubin, ketone bodies, leukocytes, urine specific gravity)-Immune/Hematological-Spleen and thymus weights, hematology (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count)-Lung/Respiratory-Lung weight-Cancer/Carcinogenesis-Heart weight-Thyroid-Thyroid weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)						
Exposure Route:	D-4 Commence	Develop [and] Mala					
Species:	Rat-Sprague	-Dawley - [fat]-Male					
HEDO ID:	607382	a Finnanae- Farent compound					
	097382						
Domain	14.1.7	Metric	Rating	Comments			
	Mettre 3.		Low	differences among groups in animal attrition or health outcomes unrelated to exposure. Urinalysis results were not reported quantitatively, although an incomplete qualitative statement was present (some parameters were changed in some groups, but no indication of the direction or magnitude of the changes). The number of animals per group was not defined in the methods, although most tables showed n=6. However, the body weight graph stated that the data represented 5 to 6 animals, and it is not clear why some of the animals were missing or which groups had 5 or 6 animals.			
Domain 5: Exposure M	lethode Sensitiv	vity					
Domain 5. Exposure M	Metric 6:	Chemical administration and characterization	Low	The test substance was identified definitively (name, CAS No., structure). A list of sources was provided, although it is unclear which substance came from which source. The purity and/or grade of test substance were not reported, and there is no indication that the purity was tested. No information was reported on the preparation or storage of the test substance. The dose was reported, but no mention of analytical verification. The route and method of exposure were reported and appropriate for the test substance, but the test volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	Low	Details of the exposure administration were incompletely reported. There is no infor- mation on the timing of the dosing, and the frequency of dosing is not explicitly stated (assuming 1x/day, 7 days/week). There is not enough information to determine if the exposures were administered consistently between treatment groups.			
Domain 6. Outagers M	and D-	aulta Diamlay					
Domain 6: Outcome M	Metric 8:	Endpoint sensitivity and specificity	Low	Only a single dose was tested, and the concentration selection was not justified. The test animal was obtained from a commercial source and were appropriate for the study. The animal numbers per group were not reported, although most tables suggested that there were 5-6 males in each group. The outcome assessment protocols were reported,			

Continued on next page ...

Medium

although there is not enough information to determine if they were evaluated consistently. The outcome methodology only partially addressed the outcome of interests as

Data were presented quantitatively along with the appropriate statistical analysis. Uri-

histopathology and functionality were not evaluated.

nalysis data was not reported.

Diethylhexyl Phthalate

Metric 9:

Results presentation

HERO ID: 697382 Table: 3 of 5

		continued from previous pa	ge			
Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). risk assessment. Journal of Toxicology and En	Comparative toxicological evalu vironmental Health. Part A: Cur	ation of phthalate diesters and metabolites in Sprague-Dawley male rats for rent Issues 72(21-22):1446-1454.			
Health Outcome(s)	Other (please specify below) (Clinical signs,	endocrine)-Clinical signs, adr	enal gland weight-Hepatic/Liver-Liver weight, serum chemistry (choles-			
and Reported	terol, triglyceride, total bilirubin, total protein	, alkaline phosphatase, glutama	te pyruvate, glutamate oxaloacetate transaminase, g-gluamyl transferase)-			
Health Effect(s):	Renal/Kidney-Kidney weight, serum chemistry	y (calcium, potassium, sodium, a	albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood,			
	pH, protein, urobilinogen, glucose, nitrite, bilir	ubin, ketone bodies, leukocytes,	urine specific gravity)-Immune/Hematological-Spleen and thymus weights,			
	hematology (red blood cell count, hemoglobin	n concentration, hematocrit, me	ean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular			
	hemoglobin concentration, platelet count, whi	te blood cell count)-Lung/Respi	ratory-Lung weight-Cancer/Carcinogenesis-Heart weight-Thyroid-Thyroid			
	weight					
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)					
Exposure Route:						
Species:	Rat-Sprague-Dawley - [rat]-Male	Rat-Sprague-Dawley - [rat]-Male				
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	697382					
Domain	Metric	Rating	Comments			
Additional Comments:	None					
Overall Quality Determination Low						

Study Citation:	Kwack, S., I	Kim, K., Kim, H., Lee, B. (2009). Comparat	tive toxicolo	gical evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for			
Health Outcome(s) and Reported Health Effect(s): Duration and	risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Other (please specify below) (Clinical signs, endocrine)-Clinical signs, adrenal gland weight-Hepatic/Liver-Liver weight, serum chemistry (choles- terol, triglyceride, total bilirubin, total protein, alkaline phosphatase, glutamate pyruvate, glutamate oxaloacetate transaminase, g-gluamyl transferase)- Renal/Kidney-Kidney weight, serum chemistry (calcium, potassium, sodium, albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood, pH, protein, urobilinogen, glucose, nitrite, bilirubin, ketone bodies, leukocytes, urine specific gravity)-Immune/Hematological-Spleen and thymus weights, hematology (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count)-Lung/Respiratory-Lung weight-Cancer/Carcinogenesis-Heart weight-Thyroid-Thyroid weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)						
Exposure Route:							
Species:	Rat-Sprague	-Dawley - [rat]-Male					
Chemical: HERO ID:	697382	I Phinalate- Parent compound					
Domain	077302	Matric	Pating	Comments			
Domain 1: Reporting O	mality	Metric	Katilig	Comments			
	Metric 1:	Reporting Quality	Medium	All of the critical information was reported, including test animal species, test substance (name, CAS. No., molecular weight, chemical structure), dose and duration of expo- sure, route, and results for at least one endpoint. Most of the important information was also reported. The test animal source, strain, age, sex, and starting body weights were reported, along with the general husbandry conditions (temperature, humidity, venti- lation, light- dark cycle, diet, water availability), although the number of animals per cage was not reported. The test animal was obtained from a commercial source and were an appropriate animal model for the study. A list of sources for the test substances was provided, although it is unclear which substances came from which sources. The purity/grade were not reported. The frequency of exposure (assumed 1/day, 7 days/week) and number of animals per exposure group (figures show 5-6 animals) were not explicitly described. The assays used to evaluate the outcomes were adequately reported.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Medium	The animals were randomly allocated to groups based on their body weight, but the specific methods were not described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes were based on use of automated/computer- driven systems, standard laboratory kits, or simple objective measures.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	Not enough information was reported to determine confounding. A negative control group was used and similarly gavaged with corn oil alone. A positive control is not required for this type of study. Food consumption was measured and similar across control and treated animals (negative results reported qualitatively). Water intake was not reported. There is no indication that there were differences in husbandry conditions between the control and treatment groups.			
Domain 4: Selective Reporting and Attrition							
Continued on next page							

PUBLIC RELEASE DRAFT May 2025

Human Health Hazard Animal Toxicology Evaluation

		conti	inued from p	previous page			
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Other (please specify below) (Clinical signs, endocrine)-Clinical signs, adrenal gland weight-Hepatic/Liver-Liver weight, serum chemistry (cholesterol, triglyceride, total bilirubin, total protein, alkaline phosphatase, glutamate pyruvate, glutamate oxaloacetate transaminase, g-gluamyl transferase)-Renal/Kidney-Kidney weight, serum chemistry (calcium, potassium, sodium, albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood, pH, protein, urobilinogen, glucose, nitrite, bilirubin, ketone bodies, leukocytes, urine specific gravity)-Immune/Hematological-Spleen and thymus weights, hematology (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count)-Lung/Respiratory-Lung weight-Cancer/Carcinogenesis-Heart weight-Thyroid-Thyroid weight Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)						
Exposure Route:	D-4 Commence	Develop [and] Mala					
Species:	Rat-Sprague	-Dawley - [fat]-Male					
HEDO ID:	607382	a Finnanae- Farent compound					
	097382						
Domain	14.1.7	Metric	Rating	Comments			
	Mettre 3.		Low	differences among groups in animal attrition or health outcomes unrelated to exposure. Urinalysis results were not reported quantitatively, although an incomplete qualitative statement was present (some parameters were changed in some groups, but no indication of the direction or magnitude of the changes). The number of animals per group was not defined in the methods, although most tables showed n=6. However, the body weight graph stated that the data represented 5 to 6 animals, and it is not clear why some of the animals were missing or which groups had 5 or 6 animals.			
Domain 5: Exposure M	lethods Sensitiv	vity					
Domain 5. Exposure M	Metric 6:	Chemical administration and characterization	Low	The test substance was identified definitively (name, CAS No., structure). A list of sources was provided, although it is unclear which substance came from which source. The purity and/or grade of test substance were not reported, and there is no indication that the purity was tested. No information was reported on the preparation or storage of the test substance. The dose was reported, but no mention of analytical verification. The route and method of exposure were reported and appropriate for the test substance, but the test volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	Low	Details of the exposure administration were incompletely reported. There is no infor- mation on the timing of the dosing, and the frequency of dosing is not explicitly stated (assuming 1x/day, 7 days/week). There is not enough information to determine if the exposures were administered consistently between treatment groups.			
Domain 6. Outagers M	and D-	aulta Diamlay					
Domain 6: Outcome M	Metric 8:	Endpoint sensitivity and specificity	Low	Only a single dose was tested, and the concentration selection was not justified. The test animal was obtained from a commercial source and were appropriate for the study. The animal numbers per group were not reported, although most tables suggested that there were 5-6 males in each group. The outcome assessment protocols were reported,			

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Medium

although there is not enough information to determine if they were evaluated consistently. The outcome methodology only partially addressed the outcome of interests as

Data were presented quantitatively along with the appropriate statistical analysis. Uri-

histopathology and functionality were not evaluated.

nalysis data was not reported.

Diethylhexyl Phthalate

Metric 9:

Results presentation

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697382 Table: 4 of 5

	continued from previous page
Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for
-	risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s)	Other (please specify below) (Clinical signs, endocrine)-Clinical signs, adrenal gland weight-Hepatic/Liver-Liver weight, serum chemistry (choles-
and Reported	terol, triglyceride, total bilirubin, total protein, alkaline phosphatase, glutamate pyruvate, glutamate oxaloacetate transaminase, g-gluamyl transferase)-
Health Effect(s):	Renal/Kidney-Kidney weight, serum chemistry (calcium, potassium, sodium, albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood,
	pH, protein, urobilinogen, glucose, nitrite, bilirubin, ketone bodies, leukocytes, urine specific gravity)-Immune/Hematological-Spleen and thymus weights,
	hematology (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular

Health Effect(s):	Renal/Kidney-Kidney weight, serum chemi pH, protein, urobilinogen, glucose, nitrite, b hematology (red blood cell count, hemogle hemoglobin concentration, platelet count, v weight	stry (calcium, potassium, sodium, ilirubin, ketone bodies, leukocytes, obin concentration, hematocrit, mo white blood cell count)-Lung/Resp	albumin, blood urea nitrogen, creatinine, glucose), urinalysis (occult blood, urine specific gravity)-Immune/Hematological-Spleen and thymus weights, ean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular iratory-Lung weight-Cancer/Carcinogenesis-Heart weight-Thyroid-Thyroid
Duration and	Oral-Gavage-Duration: Short-term (>1-30	days)-1-4-week(s)	
Exposure Route:			
Species:	Rat-Sprague-Dawley - [rat]-Male		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	697382		
Domain	Metric	Rating	Comments
Additional Comments:	None		
Overall Qualit	y Determination	Low	

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Reproductive/Developmental-Testis and epididymis weights, sperm count and motility Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s) Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound 697382 			
Domain 1: Reporting O	nality	withit	Katilig	Comments
	Metric 1:	Reporting Quality	Medium	All of the critical information was reported, including test animal species, test substance (name, CAS. No., molecular weight, chemical structure), dose and duration of expo- sure, route, and results for at least one endpoint. Most of the important information was also reported. The test animal source, strain, age, sex, and starting body weights were reported, along with the general husbandry conditions (temperature, humidity, venti- lation, light- dark cycle, diet, water availability), although the number of animals per cage was not reported. The test animal was obtained from a commercial source and were an appropriate animal model for the study. A list of sources for the test substances was provided, although it is unclear which substances came from which sources. The pu- rity/grade were not reported. The frequency of exposure (assumed 1/day, 7 days/week) and number of animals per exposure group (figures show 5-6 animals) were not explic- itly described. The assays used to evaluate the outcomes were adequately reported.
Domain 2: Selection and	d Performance			
	Metric 2:	Allocation	Medium	The animals were randomly allocated to groups based on their body weight, but the specific methods were not described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes were based on use of automated/computer-driven systems, standard laboratory kits, or simple objective measures.
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Medium	Not enough information was reported to determine confounding. A negative control group was used and similarly gavaged with corn oil alone. A positive control is not required for this type of study. Food consumption was measured and similar across control and treated animals (negative results reported qualitatively). Water intake was not reported. There is no indication that there were differences in husbandry conditions between the control and treatment groups.
Domain 4: Selective Re	porting and At	trition		
		Contin	ued on next pa	ge

		cont	inued from previ	ous page	
Study Citation: Health Outcome(s) and Reported	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454. Reproductive/Developmental-Testis and epididymis weights, sperm count and motility				
Duration and	Oral-Gavan	e-Duration: Short-term (>1-30 days)-1-4-w	veek(s)		
Exposure Route:	Ofal-Oavag	(>1-50 days)-1-4-v	VCCK(S)		
Species:	Rat-Sprague	e-Dawley - [rat]-Male			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	697382				
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Low	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure. Urinalysis results were not reported quantitatively, although an incomplete qualitative statement was present (some parameters were changed in some groups, but no indication of the direction or magnitude of the changes). The number of animals per group was not defined in the methods, although most tables showed n=6. However, the body weight graph stated that the data represented 5 to 6 animals, and it is not clear why some of the animals were missing or which groups had 5 or 6 animals.	
Domain 5: Exposure M	Iethods Sensitiv	vity			
	Metric 6:	Chemical administration and characterization	Low	The test substance was identified definitively (name, CAS No., structure). A list of sources was provided, although it is unclear which substance came from which source. The purity and/or grade of test substance were not reported, and there is no indication that the purity was tested. No information was reported on the preparation or storage of the test substance. The dose was reported, but no mention of analytical verification. The route and method of exposure were reported and appropriate for the test substance, but the test volume was not reported.	
	Metric 7:	Exposure timing, frequency, and duration	Low	Details of the exposure administration were incompletely reported. There is no infor- mation on the timing of the dosing, and the frequency of dosing is not explicitly stated (assuming 1x/day, 7 days/week). There is not enough information to determine if the exposures were administered consistently between treatment groups.	
Domain 6: Outaama M	languras and Da	pulte Dieplay			
Domain of Outcome M	Metric 8:	Endpoint sensitivity and specificity	Medium	Only a single dose was tested, and the concentration selection was not justified. The test animal was obtained from a commercial source and were appropriate for the study. The animal numbers per group were not reported, although most tables suggested that there were 5-6 males in each group. The outcome assessment protocols were reported, although there is not enough information to determine if they were evaluated consistently. The outcome methodology addressed the intended outcome.	
	Metric 9:	Results presentation	High	Data were presented quantitatively along with the appropriate statistical analysis.	
Additional Comments:	None				
Overall Quali	ity Deteri	mination	Medium		

Study Citation:	Lee, B. M., I	Koo, H. J. (2007). Hershberger assay for antia	ndrogenic effec	cts of phthalates. Journal of Toxicology and Environmental Health, Part A: Current		
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	Issues 70(15 Nutritional/I Reproductiv ani/bulbocav Oral-Gavage Rat-Sprague Diethylbeyy	Issues 70(15-16):1365-1370. Nutritional/Metabolic-Body weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Other (please specify below) (Endocrine)-Adrenal weight- Reproductive/Developmental-The following 5 tissues were weighed: testes, ventral prostates, combined seminal vesicles and coagulating glands, levator ani/bulbocavernosus (LABC), and Cowper's gland.Serum testosterone and luteinizing hormone Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s) Rat-Sprague-Dawley - [rat]-Male				
HERO ID:	673292					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was reported along with the source. Purity was reported to be \geq 98% for DEHP, DBP and BBP; purity not reported for DINP, or DIDP. Test animals species, strain, sex, age, initial body weight and source were reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Number of animals housed per cage were not reported. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups was provided. No other methods to control for modifying factors across groups were noted.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, body weight, organ weights, serum hormone levels).		
Domain 3: Confounding	. / Variable Co	atrol				
	Metric 4:	Confounding / Variable Control	Medium	Husbandry conditions were reported and similar between groups. Negative and posi- tive control groups were included and responses were appropriate. Food intake was not reported, however body weight was not different between the groups. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which have been linking to developmental and reproductive health problems. This could potentially confound results, although if control animals were exposed to the same levels, this may not sub- stantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again plastic bottles could leach phthalates that could confound re- sults.		
Domain 4: Selective Rea	porting and At	rition				
	Metric 5:	Selective Reporting and Attrition	High	Study reported no animals died and there is no indication of health effects (no clinical signs were seen).		
Domain 5: Exposure Me	ethods Sensitiv	ity				
		Contin	ued on next pa	ige		

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Diethylhexyl Phthalate

HERO ID: 673292 Table: 1 of 2

			nueu moni previ	lous page		
Study Citation:	Lee, B. M., Koo, H. J. (2007). Hershberger assay for antiandrogenic effects of phthalates. Journal of Toxicology and Environmental Health, Part A: Current Issues 70(15-16):1365-1370					
Health Outcome(s)	Nutritional/	Metabolic-Body weight-Hepatic/Liver-Live	r weight-Renal/k	Kidney-Kidney weight-Other (please specify below) (Endocrine)-Adrenal weight-		
and Reported	Reproductiv	ve/Developmental-The following 5 tissues v	vere weighed: te	stes, ventral prostates, combined seminal vesicles and coagulating glands, levator		
Health Effect(s):	ani/bulboca	vernosus (LABC), and Cowper's gland.Seru	im testosterone a	nd luteinizing hormone		
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-7-10-	day(s)	C		
Exposure Route:	e					
Species:	Rat-Sprague	Rat-Sprague-Dawley - [rat]-Male				
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	673292	-				
Domain		Metric	Rating	Comments		
	Metric 6:	Chemical administration and	Low	Purity was reported to be ≥98% for DEHP, DBP and BBP; purity not reported for		
		characterization		DINP, or DIDP. Source of test substance was reported. Gavage volume was not reported. Preparation and storage of test substance were not fully reported.		
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure duration, timing and frequency was consistent with OECD guidelines 441 for Hershberger Bioassay.		
Domain 6: Outcome Me	asures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Endpoints evaluated were in agreement with OECD guidelines 441 for Hershberger Bioassay.		
	Metric 9:	Results presentation	High	Results were fully reported with means +/- SD. Statistics were appropriate.		
Additional Comments:	None					

Study Citation:	Lee, B. M., Koo, H. J. (2007). Hershberger assay for antiandrogenic effects of phthalates. Journal of Toxicology and Environmental Health, Part A: Current			
Health Outcome(s) and Reported	Other (please	-10):1365-1370. e specify below) (Clinical signs)-Clinical sig	ns	
Duration and Exposure Route:	Oral-Gavage	-Duration: Short-term (>1-30 days)-7-10-da	ıy(s)	
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexyl 673292	-Dawley - [rat]-Male Phthalate- Parent compound		
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was reported along with the source. Purity was reported to be \geq 98% for DEHP, DBP and BBP; purity not reported for DINP, or DIDP. Test animals species, strain, sex, age, initial body weight and source were reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Number of animals housed per cage were not reported. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups was provided. No other methods to control for modifying factors across groups were noted
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported to assess clinical signs of toxicity.
Domain 3: Confounding	a / Variable Cor	atral		
	Metric 4:	Confounding / Variable Control	Medium	Husbandry conditions were reported and similar between groups. Negative and posi- tive control groups were included and responses were appropriate. Food intake was not reported, however body weight was not different between the groups. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which have been linking to developmental and reproductive health problems. This could potentially confound results, although if control animals were exposed to the same levels, this may not sub- stantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again plastic bottles could leach phthalates that could confound re- sults.
Domain 4: Selective Re	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	Study reported no animals died and there is no indication of health effects (no clinical signs were seen).
Domain 5: Exposure Me	ethods Sensitiv Metric 6:	ity Chemical administration and characterization	Low	Purity was reported to be \geq 98% for DEHP, DBP and BBP; purity not reported for DINP, or DIDP. Source of test substance was reported. Gavage volume was not reported. Preparation and storage of test substance were not fully reported.
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Study Citation:	Lee, B. M., Issues 70(1;	Lee, B. M., Koo, H. J. (2007). Hershberger assay for antiandrogenic effects of phthalates. Journal of Toxicology and Environmental Health, Part A: Current Issues 70(15-16):1365-1370.					
Health Outcome(s)	Other (pleas	se specify below) (Clinical signs)-Clinical	signs				
and Reported							
Health Effect(s):							
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-7-10	-day(s)				
Exposure Route:							
Species:	Rat-Sprague	Rat-Sprague-Dawley - [rat]-Male					
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	673292						
Domain		Metric	Rating	Comments			
	Metric 7:	Exposure timing, frequency, and	High	Exposure duration, timing and frequency was consistent with OECD guidelines 441 for			
		duration		Hershberger Bioassay.			
Domain 6: Outcome Me	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	Endpoints evaluated were in agreement with OECD guidelines 441 for Hershberger Bioassay.			
	Metric 9:	Results presentation	Medium	Clinical signs were reported as negative in text.			
Additional Comments:	None						
Overall Qualit	ty Deteri	mination	Medium				

Study Citation:	Ma, M., Ko ethylhexyl)p	ondo, T., Ban, S., Umemura, T., Kurahash hthalate affects the onset of puberty and post	ni, N., Takeda, tpubertal reprod	M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- uctive functions. Toxicological Sciences 93(1):164-171.			
and Reported	and uterus of	Reproductive/Developmental-Serum hormones (FSH, LH, testosterone, estradiol); gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary and uterus organ weights, day of vaginal opening					
Health Effect(s): Duration and	Inhalation-V	apor-Duration: Short-term (>1-30 days)					
Exposure Route:							
Species: Chemical:	Rat-Other (V	Vistar-Imamicni)-Female					
HERO ID:	674395	Thinade Tarent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.			
Domain 2. Calcotion on	d Daufauman aa						
Domain 2. Selection and	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.			
Domain 3: Confounding	v / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.			
Domain 4: Salaativa Da	norting and At	trition					
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.			
Domain 5: Exposure M	ethods Sensitiv	ity					
		Contin	ued on next pa	ge			
				<u> </u>			

PUBLIC RELEASE DRAFT May 2025

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 1 of 6

	continued from previous page
Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Reproductive/Developmental-Serum hormones (FSH, LH, testosterone, estradiol); gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary
and Reported	and uterus organ weights, day of vaginal opening
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

	Meule	Rating	Comments
Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome Measures and Re	esults Display		
Domain 6: Outcome Measures and Re Metric 8:	esults Display Endpoint sensitivity and specificity	High	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the end- points were assessed after two durations of exposure for comparison. The study au- thors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine- disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). The authors adequately justified the end- points assessed and they were sensitive to the outcomes of interest. The dose concen- trations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were sampled for the endpoints described.

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Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
	ethylhexyl)phthalate affects the onset of puber	ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.				
Health Outcome(s)	Reproductive/Developmental-Serum normone	s (FSH, LH, testosterone, estradiol); gene	expression in ovaries (real-time RI-PCR), estrous cyclicity, ovary			
and Reported	and uterus organ weights, day of vaginal open	ing				
Health Effect(s):						
Duration and	Inhalation-Vapor-Duration: Short-term (>1-3	0 days)				
Exposure Route:						
Species:	Rat-Other (Wistar-Imamichi)-Female					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	674395					
Domain	Metric	Rating	Comments			

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Diethylhexyl]	Phthalate
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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-				
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights- Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs				
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)				
Exposure Route:					
Species:	Rat-Other (V	Vistar-Imamichi)-Female			
HERO ID:	674395	r ritialate- raient compound			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.	
Domain 3: Confounding	y / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.	
Domain 4: Selective Rep	porting and Att	rition			
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.	
Domain 5: Exposure Me	ethods Sensitiv	ity			
		Contin	ued on next pa	ge	

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-
Health Outcome(s)	ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-1/1. Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome Measures and Ro	esults Display		
Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the end- points were assessed after two durations of exposure for comparison. The study au- thors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine- disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thy- roid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive method to identify organ-specific toxicity, but the lung, liver, and kidney were not the focus of this study, and the lack of other assessments on these organs is not considered to be a deficiency. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sampled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results that animals showed no signs of toxicity.
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Diethylhexyl Phthalate

			. continued from previo	us page	
Study Citation:	Ma, M., Ko ethylhexyl)p	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylbexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.			
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-				
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs				
Health Effect(s):					
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)				
Exposure Route:					
Species:	Rat-Other (W	Vistar-Imamichi)-Female			
Chemical:	Diethylhexy	Phthalate- Parent compound			
HERO ID:	674395				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means \pm SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.	
Additional Comments:	None				

Overall Quality Determination

Medium

Diethylhexyl]	Phthalate
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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-				
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights- Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs				
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)				
Exposure Route:					
Species:	Rat-Other (V	Vistar-Imamichi)-Female			
HERO ID:	674395	r ritialate- raient compound			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.	
Domain 3: Confounding	y / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.	
Domain 4: Selective Rep	porting and Att	rition			
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.	
Domain 5: Exposure Me	ethods Sensitiv	ity			
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Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome Measures and Re	esults Display		
Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive method to identify organ-specific toxicity, but the lung, liver, and kidney were not the focus of this study, and the lack of other assessments on these organs is not considered to be a deficiency. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sampled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results that animals showed no signs of toxicity.
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Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.				
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-				
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs				
Health Effect(s):					
Duration and	Inhalation-V	/apor-Duration: Short-term (>1-30 da	ays)		
Exposure Route:					
Species:	Rat-Other (V	Wistar-Imamichi)-Female			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	674395				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means \pm SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.	
Additional Comments:	None				

Overall Quality Determination

Medium

Diethylhexyl Phthalate	
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Study Citation:	Ma, M., Ko	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights- Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs						
Duration and	Inhalation-V	Inhalation-Vapor-Duration: Short-term (>1-30 days)					
Exposure Route:							
Species:	Rat-Other (V	Vistar-Imamichi)-Female					
HERO ID:	674395	r ritialate- ratent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Qu	uality						
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.			
Domain 3: Confounding	y / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.			
Domain 4: Selective Rep	Domain 4: Selective Reporting and Attrition						
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.			
Domain 5: Exposure Me	ethods Sensitiv	ity					
		Contin	ued on next pa	ge			

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome Measures and Re	sults Display		
Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive method to identify organ-specific toxicity, but the lung, liver, and kidney were not the focus of this study, and the lack of other assessments on these organs is not considered to be a deficiency. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sampled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results that animals showed no signs of toxicity.
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Diethylhexyl Phthalate

continued from previous page					
Study Citation:	Ma, M., Ko ethylhexyl)r	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylbexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.			
Health Outcome(s)	Nutritional/I	Metabolic-Body weights, food a	nd water intake-Hepa	tic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-	
and Reported	Lung/Respir	ratory-Lung weights-Other (please sp	ecify below) (Clinical si	gns)-Undefined clinical signs	
Health Effect(s):					
Duration and	Inhalation-V	apor-Duration: Short-term (>1-30 da	ays)		
Exposure Route:					
Species:	Rat-Other (V	Wistar-Imamichi)-Female			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	674395				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means \pm SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.	
Additional Comments:	None				

Overall Quality Determination

Medium

Diethylhexyl Phthalate	
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Study Citation:	Ma, M., Ko	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights- Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs						
Duration and	Inhalation-V	Inhalation-Vapor-Duration: Short-term (>1-30 days)					
Exposure Route:							
Species:	Rat-Other (V	Vistar-Imamichi)-Female					
HERO ID:	674395	r ritialate- raient compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Qu	uality						
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.			
Domain 3: Confounding	y / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.			
Domain 4: Selective Rep	Domain 4: Selective Reporting and Attrition						
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.			
Domain 5: Exposure Me	ethods Sensitiv	ity					
		Contin	ued on next pa	ge			

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome Measures and R	esults Display		
Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the end- points were assessed after two durations of exposure for comparison. The study au- thors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine- disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thy- roid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive method to identify organ-specific toxicity, but the lung, liver, and kidney were not the focus of this study, and the lack of other assessments on these organs is not considered to be a deficiency. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sampled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results that animals showed no signs of toxicity.
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Diethylhexyl Phthalate

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Study Citation:	Ma, M., Ko ethylhexyl)p	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.				
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-					
and Reported	Lung/Respir	atory-Lung weights-Other (please sp	ecify below) (Clinical si	gns)-Undefined clinical signs		
Health Effect(s):						
Duration and	Inhalation-V	Vapor-Duration: Short-term (>1-30 d	ays)			
Exposure Route:						
Species:	Rat-Other (V	Wistar-Imamichi)-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674395					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means \pm SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.		
Additional Comments:	None					

Overall Quality Determination

Medium

Diethylhexyl Phthalate	
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Study Citation:	Ma, M., Ko	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights- Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs						
Duration and	Inhalation-V	Inhalation-Vapor-Duration: Short-term (>1-30 days)					
Exposure Route:							
Species:	Rat-Other (V	Vistar-Imamichi)-Female					
HERO ID:	674395	r ritialate- raient compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Qu	uality						
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.			
Domain 3: Confounding	y / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.			
Domain 4: Selective Rep	Domain 4: Selective Reporting and Attrition						
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.			
Domain 5: Exposure Me	ethods Sensitiv	ity					
		Contin	ued on next pa	ge			

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-			
Health Outcome(s)	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-			
and Reported	Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs			
Health Effect(s):				
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 days)			
Exposure Route:				
Species:	Rat-Other (Wistar-Imamichi)-Female			
Chemical:	Diethylhexyl Phthalate- Parent compound			
HERO ID:	674395			

Domain	Metric	Rating	Comments		
Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.		
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.		
Domain 6: Outcome Measures and Results Display					
Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive method to identify organ-specific toxicity, but the lung, liver, and kidney were not the focus of this study, and the lack of other assessments on these organs is not considered to be a deficiency. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sampled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results that animals showed no signs of toxicity.		
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Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylbexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171			
Health Outcome(s)	Nutritional/Metabolic-Body weights, food an	nd water intake-Hepa	tic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-	
and Reported	Lung/Respiratory-Lung weights-Other (please spe	ecify below) (Clinical si	gns)-Undefined clinical signs	
Health Effect(s):				
Duration and	Inhalation-Vapor-Duration: Short-term (>1-30 da	ys)		
Exposure Route:				
Species:	Rat-Other (Wistar-Imamichi)-Female			
Chemical:	Diethylhexyl Phthalate- Parent compound			
HERO ID:	674395			
Domain	Metric	Rating	Comments	
	Metric 9: Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means \pm SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.	
Additional Comments:	None			

Overall Quality Determination

Medium

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Santacruz-Márquez, R., Safar, A. M., Laws, M. J., Meling, D. D., Liu, Z., Kumar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The effects of short-term and long-term phthalate exposures on ovarian follicle growth dynamics and hormone levels in female mice [†] . Biology of Reproduction 110(1):198-210. Reproductive/Developmental-Ovary histopathology, serum hormones (progesterone, testosterone, estradiol, FSH, LH), and gene expression in ovarian tissue-Other (please specify below) (Endocrine)-Gene expression in pituitary tissue Oral-Diet-Duration: Short-term (>1-30 days)-7-1-month(s) Mouse-CD-1 - [mouse]-Female Diethylhexyl Phthalate- Parent compound 11784618			
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified as di(2-ethylhexyl)phthalate. No CASRN was pro- vided. The test substance was sourced from Sigma-Aldrich (St. Louis, MO). Test animal species, strain, sex, age, and source were reported. It was not specified whether mice were virgins (33 days old at purchase), and Initial body weights were not reported. Hus- bandry conditions (temperature, humidity, and light cycle) were not reported. Animals were housed 3/cage. Feed and water were available ad libitum. Dose levels (ppm), du- ration, and route of exposure were reported; however, the number of animals/group was not clearly stated, but sample sizes for each endpoint were specified. Target concentra- tions were reported; however, actual doses were not. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and	1 Performance			
	Metric 2:	Allocation	Low	Allocation methods were not reported.
	Metric 3:	Observational Bias / Blinding Changes	High	Humans that were counting the follicle populations were blinded to treatments. Blinding for other measures was not reported; however, the endpoints evaluated were either not subjective in nature or consisted of histopathology.
Domain 3: Confounding	g / Variable Cor	ntrol		
	/ Variable Control Metric 4: Confounding / Variable Control Low Body weight and food intake were not reported in a study with dietary exposures. The authors cited a previous study by the same group that showed exposure to the test substance via the diet did not affect body weight or food consumption. A negative control group was included (rodent chow with 7% corn oil) and responses were appropriate fo negative controls. Housing conditions (e.g., bedding, RO water, animals per cage) wer consistent across groups but animal husbandry details (temperature, humidity etc.,) we not reported. The study did not indicate whether measures were taken to reduce exposure to plasticizers from bedding, feed, or equipment (e.g., water dispensers). No testin for contaminates was described and the study was assessing endocrine disruption. The study noted that animals were sacrificed in diestrus. No further details were provided and it is unclear whether sacrifices were conducted on the same day.			

Domain 4: Selective Reporting and Attrition

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 11784618 Table: 1 of 1

		conti	nued from prev	ious page		
Study Citation:	Santacruz-M effects of she 110(1):198-7	Santacruz-Márquez, R., Safar, A. M., Laws, M. J., Meling, D. D., Liu, Z., Kumar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The effects of short-term and long-term phthalate exposures on ovarian follicle growth dynamics and hormone levels in female mice [†] . Biology of Reproduction 110(1):108-210				
Health Outcome(s)	Reproductiv	e/Developmental-Ovary histopathology, se	erum hormones (progesterone, testosterone, estradiol, FSH, LH), and gene expression in ovarian		
and Reported	tissue-Other	(please specify below) (Endocrine)-Gene	expression in pitu	itarv tissue		
Health Effect(s):			I I I I			
Duration and	Oral-Diet-D	uration: Short-term (>1-30 days)-7-1-mon	th(s)			
Exposure Route:	ofui bier b		un(b)			
Snecies.	Mouse-CD-	1 - [mouse]-Female				
Chemical.	Diethylhexy	Philase Parent compound				
HERO ID:	11784618	Trininalate Tarent compound				
Domain	11/01010	Metric	Rating	Comments		
Domain	Matric 5:	Selective Reporting and Attrition	Low	Data ware reported for almost all outcomes. The methods stated that nituitary clands		
	Welle 3.	Secence Reporting and Autobi	Low	were collected for analysis of pituitary gene expression. It is unclear from the text whether pituitaries were collected from both short-term and chronic duration experi- ments, but results were only reported for the long-term exposure groups. Insufficient information was provided to assess attrition. The number of animals per group was not specified in the methods and sample sizes varied from 3-8 per endpoint and in some cases, numbers varied from 4-6 within an endpoint. No justification for the differences in sample sizes was provided and it is unclear if this represents selective reporting.		
Domain 5: Exposure M	Iethods Sensitiv Metric 6:	ity Chemical administration and	Low	The purity of the test substance was not reported; however, the Ssource was specified (Sigma-Aldrich and purities on the supplier website were all $>98\%$. Certificates of		
		characterization		(Signa-Adden, and purifies on the supplier website were an >95%. Certificates of analysis are available from the supplier upon request; the test substance was not analyt- ically verified by the performing laboratory. Envigo Tekland was supplied with the test substance in corn oil, and the diets were prepared (no additional details were provided). Target test concentrations in food (ppm) were reported; there is no indication that anal- ysis was done. The authors provided "rough equivalents" in mg/kg-day; however, it was not specified how these estimates were made - Only target concentrations were reported; no analysis was done. No feed intake or body weights were recorded and ADD was not calculated. Dietary exposure was selected to mimic human exposure.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency were appropriate for the study type and the out- comes of interest. The durations were justified by the study authors.		
Domain 6: Outcome M	easures and Re	sults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. A limited number of endpoints were assessed but were in		
	Medie 6.		mgn	line with the specified goals of the study. Outcome methodologies were reported and were sensitive to the outcomes of interest. The test animal species was appropriate and obtained from a commercial source. The exposure concentrations were based on a previously published rationale and were meant to fall within daily human exposure, infant exposure, and occupational exposure. Sample sizes varied across and within endpoints (see Metric 4) but were sufficient to allow for statistical analysis. For several endpoints, the authors noted that inter-assay coefficients of variability were <10%.		
	Metric 9:	Results presentation	High	Results were described in the text and data were presented graphically showing means \pm SEM. Individual animal data were also included. Statistical analysis methods were described and were appropriate, and statistical significance was noted in graphs.		

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Diethylhexyl Phthalate

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Study Citation:	Santacruz-Márquez, R., Safar, A. M., Laws, M effects of short-term and long-term phthalate exp 110(1):198-210.	I. J., Meling, D. D., Liu, Z., Kuma posures on ovarian follicle growth dy	ar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The vnamics and hormone levels in female mice [†] . Biology of Reproduction
Health Outcome(s)	Reproductive/Developmental-Ovary histopathol	ogy, serum hormones (progesterone	e, testosterone, estradiol, FSH, LH), and gene expression in ovarian
and Reported	tissue-Other (please specify below) (Endocrine)-	-Gene expression in pituitary tissue	
Health Effect(s):			
Duration and	Oral-Diet-Duration: Short-term (>1-30 days)-7-	-1-month(s)	
Exposure Route:			
Species:	Mouse-CD-1 - [mouse]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	11784618		
Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination

Medium

Study Citation: Health Outcome(s) and Reported Health Effect(s):	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104. Nutritional/Metabolic-Body weight				
Duration and	Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-15-day	y(s)		
Exposure Route:	D				
Species:	Rat-Sprague	-Dawley - [rat]-Male			
HERO ID:	697420	r r nulaiate- r arent compound			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, num- ber of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and num- ber of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to sub- stantially impact the interpretation of the results.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.	
Domain 3: Confounding	g / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint(s) of interest.	
Domain 4: Selective Rep	porting and At	trition			

Diethylhexyl Phthalate

		cont	inued from previ	ous page		
Study Citation:	Vo, B., T.T.	., Jung, E. M., Dang, V. H., Yoo, Y. M., G in the testis of immature male rats. Reprodu	Choi, K. C., Yu, I	F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene 1 Endocrinology 7:104.		
Health Outcome(s) and Reported	Nutritional/	Nutritional/Metabolic-Body weight				
Health Effect(s):						
Duration and	Oral-Gavag	e-Duration: Short-term (>1-30 days)-7-15-	-day(s)			
Exposure Route:	-					
Species:	Rat-Spragu	e-Dawley - [rat]-Male				
Chemical:	Diethylhexy	yl Phthalate- Parent compound				
HERO ID:	697420					
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the meth- ods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. Overall, these omissions are not expected to significantly im- pact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.		
Domain 5: Exposure N	lethods Sensitiv	vity				
Domain 5: Exposure iv	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.		
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.		
Domain 6 [,] Outcome N	leasures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoint of interest. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The sample size (4 animals/group) was small but sufficient to perform statistics. The frequency of body weight measurements was reported and protocols were consistent across groups.		
	Metric 9:	Results presentation	Medium	Quantitative data (mean \pm SD, represented graphically) were provided for body weights measured at necropsy. Statistical methods were described and were appropriate. Body weight data collected during the exposure period were not reported.		
Additional Comments:	None					

Overall Quality Determination

Medium

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Diethylhexyl Phthalate

HERO ID: 697420 Table: 1 of 4

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Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y expression in the testis of immature male rats. R	M., Choi, K. C., Yu, F. H., Jeung eproductive Biology and Endocrinol	E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene ogy 7:104.		
Health Outcome(s)	Nutritional/Metabolic-Body weight				
and Reported					
Health Effect(s):					
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)				
Exposure Route:					
Species:	Rat-Sprague-Dawley - [rat]-Male				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	697420				
Domain	Metric	Rating	Comments		

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104. Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)				
Exposure Route: Species:	Rat-Sprague	-Dawley - [rat]-Male			
Chemical: HERO ID:	Diethylhexy 697420	1 Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, num- ber of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and num- ber of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to sub- stantially impact the interpretation of the results.	
Domain 2: Selection an	d Performance Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other	
	Wether 2.	A mocation	Low	methods to control for modifying factors across groups were noted.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures. Blinding was not reported for clinical signs.	
Domain 3: Confoundin	g / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint(s) of interest.	
Domain 4: Selective Re	porting and At	trition			
		Cont	inued on next page		

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Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 697420 Table: 2 of 4

		C	ontinued from previous	page	
Study Citation: Health Outcome(s) and Reported	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104. Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior				
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-15-da	ay(s)		
Species:	Rat-Sprague	e-Dawley - [rat]-Male			
Chemical: HERO ID:	Diethylhexy 697420	l Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the meth- ods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. Overall, these omissions are not expected to significantly im- pact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.	
Domain 5: Exposure M	ethods Sensitiv	vity			
Domain 5. Exposure in	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.	
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.	
Domain 6: Outcome M	essures and P e	sulte Display			
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoints. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The sample size (4 animals/group) was small and may reduce statistical power. The frequency of animal observations was reported (daily), but no additional methodological details for observing clinical signs were provided (e.g., cage-side; detailed clinical). This is not expected to have a significant impact on the study results.	
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for observed clinical signs and	

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697420 Table: 2 of 4

	continued from previous page					
Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. expression in the testis of immature male rats. Reference of the second s	. M., Choi, K. C., Yu, F. H., Jeung, E. B. (200 eproductive Biology and Endocrinology 7:104.	09). Di-(2 ethylhexyl) phthalate and flutamide alter gene			
Health Outcome(s)	Other (please specify below) (Clinical signs)-Ob	served clinical signs-Neurological/Behavioral-A	Abnormal behavior			
and Reported						
Health Effect(s):						
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days))-7-15-day(s)				
Exposure Route:						
Species:	Rat-Sprague-Dawley - [rat]-Male					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	697420					
Domain	Metric	Rating	Comments			
Overall Quali	ity Determination	Uninformative				

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104. Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s) Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound 697420 			
Domain	1.	Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, num- ber of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and num- ber of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to sub- stantially impact the interpretation of the results.
Domain 2: Selection and	d Performance			
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures. Blinding was not reported for clinical signs.
Domain 3: Confounding	a / Variable Cor	atrol		
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint(s) of interest.
Domain 4: Selective Re	porting and Att	rition		

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697420 Table: 3 of 4

		c	ontinued from previous	page	
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104. Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior				
Duration and Exposure Route:	Oral-Gavage	e-Duration: Short-term (>1-30 days)-7-15-d	ay(s)		
Species:	Rat-Sprague	e-Dawley - [rat]-Male			
Chemical: HERO ID:	Diethylhexy 697420	l Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the meth- ods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. Overall, these omissions are not expected to significantly im- pact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.	
Domain 5: Exposure M	lethods Sensitiv	vity			
Domani 5. Exposure iv	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.	
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.	
Domain 6: Outcome N	leasures and Re	esults Display			
2 ontain of Outcome it.	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoints. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The sample size (4 animals/group) was small and may reduce statistical power. The frequency of animal observations was reported (daily), but no additional methodological details for observing clinical signs were provided (e.g., cage-side; detailed clinical). This is not expected to have a significant impact on the study results.	
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for observed clinical signs and abnormal behaviors.	
Additional Comments:	None				

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		continued from previous page	
Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo expression in the testis of immature male rat	o, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (200 s. Reproductive Biology and Endocrinology 7:104	9). Di-(2 ethylhexyl) phthalate and flutamide alter gene
Health Outcome(s) and Reported	Other (please specify below) (Clinical signs)	-Observed clinical signs-Neurological/Behavioral-A	bnormal behavior
Health Effect(s):			
Duration and	Oral-Gavage-Duration: Short-term (>1-30 d	ays)-7-15-day(s)	
Species:	Rat-Sprague-Dawley - [rat]-Male		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	697420		
Domain	Metric	Rating	Comments
Overall Qual	ity Determination	Uninformative	

Diethy	lhexvl	Phthalate
Dictin	1110A y 1	1 minutate

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Vo, B., T.T., expression in Reproductiv hormone lev Plcd1, Lhx1 Oral-Gavage Rat-Sprague Diethylhexy 697420	, Jung, E. M., Dang, V. H., Yoo, Y. M., C n the testis of immature male rats. Reproduc e/Developmental-Organ weights (testis, ep rels; histological analysis (testes); gene exp , and Isoc1 expression by RT-PCR) e-Duration: Short-term (>1-30 days)-7-15-c -Dawley - [rat]-Male l Phthalate- Parent compound	thoi, K. C., ctive Biolog ididymis, pr pression in t day(s)	Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene y and Endocrinology 7:104. ostate, seminal vesicle); anogenital distance; circulating testosterone and luteinizing estes (37,317 genes by cDNA microarray; StAR, Cyp11a1, HSD3b1, CaBP1, Vav2,
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality			
	Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, num- ber of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and num- ber of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to sub- stantially impact the interpretation of the results.
Domain 2: Selection an	d Performance			
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or consisted of an initial histopathology review, and no secondary histopathology review was conducted.
Domain 3: Confoundin	g / Variable Co	ntrol		
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication

Domain 4: Selective Reporting and Attrition

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not described.

of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697420 Table: 4 of 4

		con	tinued from p	revious page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Vo, B., T.T. expression i Reproductiv hormone le Plcd1, Lhx1 Oral-Gavag Rat-Sprague Diethylhexy 697420	, Jung, E. M., Dang, V. H., Yoo, Y. M., n the testis of immature male rats. Repro- 'e/Developmental-Organ weights (testis, vels; histological analysis (testes); gene , and Isoc1 expression by RT-PCR) e-Duration: Short-term (>1-30 days)-7-1: e-Dawley - [rat]-Male vl Phthalate- Parent compound	Choi, K. C., ductive Biology epididymis, pr expression in t 5-day(s)	Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter generation of the second state of the second s
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the meth- ods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. The methods state that testes were collected from each group for total RNA isolation; however, results were only reported for the 100 mg/kg-day group. Overall, these omissions are not expected to significantly impact the interpreta- tion of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.
Domain 5: Exposure N	Aethods Sensitiv	vity		
·	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697420 Table: 4 of 4

Diethylhexyl Phthalate

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Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s)	Reproductive/Developmental-Organ weights (testis, epididymis, prostate, seminal vesicle); anogenital distance; circulating testosterone and luteinizing
and Reported	hormone levels; histological analysis (testes); gene expression in testes (37,317 genes by cDNA microarray; StAR, Cyp11a1, HSD3b1, CaBP1, Vav2,
Health Effect(s):	Plcd1, Lhx1, and Isoc1 expression by RT-PCR)
Duration and	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Exposure Route:	
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoints. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The number of animals (4 animals/group) was low and likely reduced the statistical power and ability to detect some changes. In addition, for gene expression and histological analyses, it is not clear how many animals were used for each. In the methods section, it is stated that "four testes were collected from each group for total RNA isolation. Other testes were fixed in Bouin's solution, paraffin-embedded, and sectioned at 5 um for histopathological examination." It is not clear if one testis was collected from each experimental animal for RNA isolation and the remaining four testes were used for histological analysis. This uncertainty on the number of animals used to generate the samples is expected to impact the interpretation of gene expression and histological findings. It is unlikely that the sample size was adequate for histopathology.
	Metric 9:	Results presentation	Low	Quantitative data (mean \pm SD, represented graphically) were provided for organ weights, anogenital distance, circulating testosterone and LH levels, and some gene ex- pression analysis in the testes. Quantitative data (fold change compared to vehicle) were provided for gene expression results of cDNA microarray analysis for the 100 mg/kg- day group. No microarray results for the low- and high-dose groups were reported. No supplemental files or links to database holdings of the array data were provided. Statisti- cal methods were described and were appropriate for the endpoints. There is a discrep- ancy regarding the anogenital distance data, the study authors state in the Results and Discussion sections that anogenital distance was significantly reduced in the 500 mg/kg bw/day group as compared to the vehicle. However, this reduction is not apparent on the graph which appears to show an increase in anogenital distance in the low and mid-dose groups, and no change from the negative control in the 500 mg/kg-day group. The sta- tistical analysis of the AGD data is also questionable. The figure suggested statistical analysis may have been mistakenly done using the positive control rather than the neg- ative control. The discrepancies in the anogenital distance results substantially impact the interpretation of that endpoint. Histopathology data were presented as representative figures instead of incidence data.
ional Comments:	None			

continued from previous page

Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple					
Health Outcome(s) and Reported Health Effect(s):	endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780. Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology): aromatase gene expression in Leydig cells					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Subchronic (>30-90 days)-7-70-	day(s)			
Species:	Rat-Long-E	vans - [rat]-Male				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	673552					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	Low	The study does not report how animals were allocated to study groups. No other meth- ods to control for modifying factors across groups were noted.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).		
Domain 3: Confounding	n / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Food and water dispensing containers were not described.		
Domain 4: Selective Re	porting and At	trition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for most prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.		
Domain 5: Exposure M	ethods Sensitiv	vity				
		Contin	ued on next pa			

Human Health Hazard Animal Toxicology Evaluation

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HERO ID: 673552 Table: 1 of 1

Diethylhexyl Phthalate	
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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780. Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells Oral-Gavage-Duration: Subchronic (>30-90 days)-7-70-day(s)					
Exposure Route:						
Species:	ies: Rat-Long-Evans - [rat]-Male					
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	673552					
Domain		Metric	Rating	Comments		
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.		
Domain 6: Outcome Me	easures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.		
	Metric 9:	Results presentation	Medium	Results for most endpoints were described in the text and data were presented in tables as means \pm standard error. Statistical tests were reported and appropriate. Histopathological data were reported as negative in the text.		
Additional Comments:	Study includ performed of evaluation c	ded 3 different durations of exposure during on different cohorts of animals (though this corresponds with the PND21-90 duration	post weaning up s is unclear and a	to adulthood. each "experiment" included different outcomes and were potentially all groups were >10). not all outcomes were evaluated for each duration. This		

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PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

Study Citation:	Gu, Y., Gao, M., Zhang, W., Yan, L., Shao, F., Zhou, J. (2021). Exposure to phthalates DEHP and DINP May lead to oxidative damage and lipid disruptions in mouse kidney. Chemosphere 271:129740.						
Health Outcome(s)	(s) Nutritional/Metabolic-Body weight-Renal/Kidney-organ weight, renal biomarkers for oxidative stress (ROS, MDA, GSH), inflammatory cytokines (TN						
and Reported	and IL-6)						
Health Effect(s):	0.1.0						
Duration and	Oral-Gavage-Duration: Subchronic (>30-90 days)-7-5-week(s)						
Exposure Route: Species:							
Chemical:	Diethylhexy	¹ Phthalate- Parent compound					
HERO ID:	7978408						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	Juality						
	Metric 1:	Reporting Quality	Medium	All critical information was reported. The chemical name (Di-isononyl phthalate, DINP or DEHP). The exposure concentration of low (0.05mg/kg bw), and high (4.8 mg/kg bw) and vehicle control (corn oil), the duration of exposure (daily for 5 weeks), and the route of exposure (gavage) were provided. The test animal species (mice), strain (ICR), sex (male), animal supplier (Charles River Co. Ltd (China)), age at the time of exposure was specified (3 week). Information on animal husbandry; temperature (20-26 C°), humidity (40%e70%), and 1:1 hours light/dark cycle were reported. Animal were houses in polypropylene cages for acclimation-14 days, glass water bottles and fed ad libitum. The endpoint evaluation methods , and initial weight of animals were not described. CASRN#, the purity was not reported.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Low	The animal were selected randomly, no indication of other methods.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	The study is considered Medium for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., , body weight).			
Domain 3: Confoundin	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A vehicle control groups was included. No effect of test substance palatability in di- etary exposure leading to differences in food consumption or body weight was reported among the study group. All animal husbandry conditions were sufficient: temperature, humidity, light/dark cycle, diet, water availability, ad libitum.			
Domain 4: Selective Re	porting and At	trition					
	Metric 5:	Selective Reporting and Attrition	High	Quantitative or qualitative results were reported for all prespecified outcomes, no animal attrition identified.			
Domain 5: Exposure M	ethods Sensitiv	vity					
	Metric 6:	Chemical administration and characterization	Low	Test substance was identified by name (DINP) and not CASRN #. Animals were divided into 3 groups at 2 dose levels and a control, however, impurities is substantial or concerning.			
		Contin	ued on next pa	nge			

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 7978408 Table: 1 of 1

		conti	inued from previ	ous page			
Study Citation:	Gu, Y., Gao, M., Zhang, W., Yan, L., Shao, F., Zhou, J. (2021). Exposure to phthalates DEHP and DINP May lead to oxidative damage and lipidomic disruptions in mouse kidney. Chemosphere 271:129740						
Health Outcome(s)	Nutritional/N	Nutritional/Metabolic-Body weight-Renal/Kidney-organ weight, renal biomarkers for oxidative stress (ROS, MDA, GSH), inflammatory cytokines (TNF-a					
and Reported	and IL-6)						
Health Effect(s):							
Duration and	Oral-Gavage	e-Duration: Subchronic (>30-90 days)-7-5	-week(s)				
Exposure Route:							
Species:	Mouse-ICR - [mouse]-Male						
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	7978408						
Domain		Metric	Rating	Comments			
	Metric 7:	Exposure timing, frequency, and duration	Medium	The timing, duration were reported , however the frequency of the exposure was not reported.			
Domain 6: Outcome Me	asures and Rea	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Low	The test animal selected, species, strain sex, life-stage (mice, ICR, 3 weeks old male) was relevant to evaluation of the outcomes. Sample size (n=8/group) and the timing of the endpoint assessment was suitable. The limitation of methodology to address the proposed outcomes (body weight) of this study was the lack of data on food intake and changes of adipose tissue which are useful in interpreting the body weight changes observed in the low dose groups.			
	Metric 9:	Results presentation	Medium	Statistical data was presented as means and SD or SEM.			
Additional Comments:	None						

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Nutritional/Metabolic-Food consumption, water consumption, and body weight Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic				
Exposure Route: Species: Chemical: HERO ID:	(>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 2000828				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Qu	aality Metric 1:	Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.	
Domain 2: Selection and Performance Metric 2: Allocation Medium Study authors state that mice were randomly allocated into study groups, method used					
				was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the interpretation of the results.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (body weight, and food and water intake).	
Domain 3: Confounding	/ Variable Cor	atrol			
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results. Polycar- bonate cages were used instead of wire cages. Food and water dispensing containers were not described.	
Domain 4: Selective Reporting and Attrition					
Continued on next page					

May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

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		con	tinued from previo	us page	
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	 Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Nutritional/Metabolic-Food consumption, water consumption, and body weight Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 				
HERO ID:	2000828				
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Low	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition.Data for body weight and food intake were not appropriately reported. Not all timepoints were reported independently and it cannot be determined which timepoint the data presented pertains to.	
Domain 5: Exposure M	ethods Sensitiv	vity			
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.	
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.	
Domain 6: Outcome M	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.	
	Metric 9:	Results presentation	Uninformative	Body weight data was reported however it is unclear which time point these data pertain to. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state ter- minal body weights were recorded for each. Table 1 reports body weight data but does not indicate which timepoint this is for. Lack of this information makes this endpoint uninformative. Mean food intake was reported however which timepoint these values pertain to or how many animals were included in the calculation were not reported. Wa- ter intake is reported as an approximation of 7 ml/day.	

Additional Comments: None

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 2000828 Table: 1 of 3

Diethylhexyl Phthalate

		continued from previous page				
Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, Nocal immunity in the testis by exposure to di	M., Qu, N., Hatayama, N., Miyaso, H., Mat -(2-ethylhexyl) phthalate (DEHP) in mice.	tsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the Journal of Reproduction and Development 59(5):485-490.			
Health Outcome(s)	Nutritional/Metabolic-Food consumption, wa	Nutritional/Metabolic-Food consumption, water consumption, and body weight				
and Reported						
Health Effect(s):						
Duration and	Oral-Diet-Duration: Short-term (>1-30 day	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic				
Exposure Route:	(>30-90 days)-7-8-week(s)					
Species:	Mouse-A/J - [mouse]-Male					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	2000828					
Domain	Metric	Rating	Comments			
Overall Quali	ity Determination	Medium				

Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490.
Health Outcome(s)	Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of
and Reported	seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase
Health Effect(s):	detection)
Duration and	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic
Exposure Route:	(>30-90 days)-7-8-week(s)
Species:	Mouse-A/J - [mouse]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000828

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.
Domain 2: Selection and Performance			
Metric 2:	Allocation	Medium	Study authors state that mice were randomly allocated into study groups, method used was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the interpretation of the results.
Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Co	ntrol		
Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and valid- ity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 2000828 Table: 2 of 3

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		conti	nued from previ	ous page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase detection) Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 2000828			
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantita- tive or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.
Domain 5: Exposure M	athods Sansitiv			
Domain 5: Exposure M	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome M	easures and Re	culte Dienlay		
Johan o. Outcome M	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Data for testis weight is not appropriately reported. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state testes weights was recorded for each timepoint. Table 1 reports testes weight data but does not indicate which timepoint this is for. Histopathology of testes was reported sufficiently with means and SD. Statistical analysis was performed and appropriate. Although testicular weight data cannot be used for this assessment, the histopathological data is adequately reported.

Additional Comments: None

Continued on next page ...

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Health Outcome(s) Reproductive/Developmental-Absolute testis weight, Histological analyses of testes: degree of spermatogenic disturbance (Johnsen's score), numbers of seminiferous tubules with vacuoles in the cytoplasm of Sertoli cells; determination of the permeability of the blood-testis-barrier ((horseradish peroxidase and Reported **Health Effect(s):** detection) **Duration and** Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic **Exposure Route:** (>30-90 days)-7-8-week(s) Species: Mouse-A/J - [mouse]-Male **Chemical:** Diethylhexyl Phthalate- Parent compound **HERO ID:** 2000828 Domain Metric Rating Comments

Overall Quality Determination

Medium

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Kitaoka, M., Hirai, S., Terayama, H., Naito, M., Qu, N., Hatayama, N., Miyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the local immunity in the testis by exposure to di-(2-ethylhexyl) phthalate (DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. Immune/Hematological-Lymphocyte infiltration into testicular interstium; Immunohistochemistry for T-cells (CD3), B- cells (CD45R/B220), macrophages (F4/80), MHC-II, IFNγ, and IL-10; mRNA expression of cytokines (IFNγ, TNFα, IL-6, and IL-10) in the testis Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s) Mouse-A/J - [mouse]-Male Diethylhexyl Phthalate- Parent compound 2000828 				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and most important information were reported in this study. The study in- cluded identification of the test substance (di-(2-ethylhexyl) phthalate), and source (Tokyo Chemical Industry); test animal characteristics (species, strain, age, sex); gen- eral animal husbandry conditions (temperature, humidity, light/dark cycle, diet, water availability); exposure methods (purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age); and endpoint evaluation methods (quantitative and qualitative). The study was lacking some important information including the starting body weights of the test animals and the number of animals per cage throughout the study. All critical information is provided and although some important information is missing, the missing information is not expected to sig- nificantly impact the study evaluation.	
Domain 2: Selection and	d Performance Metric 2:	Allocation	Medium	Study authors state that mice were randomly allocated into study groups, method used was not reported. The study authors did not provide the starting body weights of the test animals. Therefore, it could not be determined whether body weights were evenly spread out across the study groups. This could potentially substantially impact the inter-	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.	
Domain 3: Confounding	g / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received undosed feed. A posi- tive control group was not included and is not required. Animal husbandry conditions appeared to be consistent across study groups. An overall food intake was reported how- ever it is unclear what duration or how many animals were used in the calculation. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results. Polycar- bonate cages were used instead of wire cages. Food and water dispensing containers were not described.	
Domain 4: Selective Re	porting and At	trition			

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 2000828 Table: 3 of 3

Hirai, S., Terayama, H., Naito, M., Qu, N. ty in the testis by exposure to di-(2-ethylh hatological-Lymphocyte infiltration into te C-II, IFN γ , and IL-10; mRNA expression ration: Subchronic (>30-90 days)-7-8-we [mouse]-Male	., Hatayama, N., M exyl) phthalate (I sticular interstium of cytokines (IFN æk(s)	Aiyaso, H., Matsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the DEHP) in mice. Journal of Reproduction and Development 59(5):485-490. a; Immunohistochemistry for T-cells (CD3), B- cells (CD45R/B220), macrophages γ , TNF α , IL-6, and IL-10) in the testis				
ration: Subchronic (>30-90 days)-7-8-we [mouse]-Male	eek(s)					
[mouse]-Male		Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s)				
Phthalate- Parent compound						
Metric	Rating	Comments				
Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantita- tive or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.				
ty						
Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.				
Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.				
ults Display						
Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropri- ate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on exist- ing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.				
Results presentation	High	Lymphocyte infiltration was reported as means with SD. Immunohistochemistry and mRNA expression were fully reported. Statistical analysis was performed and appropriate.				
1	Results presentation	Results presentation High				

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		continued from previous page	
Study Citation:	Kitaoka, M., Hirai, S., Terayama, H., Naito, M local immunity in the testis by exposure to di-(., Qu, N., Hatayama, N., Miyaso, H., Ma (2-ethylhexyl) phthalate (DEHP) in mice	atsuno, Y., Komiyama, M., Itoh, M., Mori, C. (2013). Effects on the . Journal of Reproduction and Development 59(5):485-490.
Health Outcome(s)	Immune/Hematological-Lymphocyte infiltratio	on into testicular interstium; Immunohisto	ochemistry for T-cells (CD3), B- cells (CD45R/B220), macrophages
and Reported	(F4/80), MHC-II, IFNγ, and IL-10; mRNA exp	pression of cytokines (IFN γ , TNF α , IL-6	5, and IL-10) in the testis
Health Effect(s):			
Duration and	Oral-Diet-Duration: Subchronic (>30-90 days	s)-7-8-week(s)	
Exposure Route:	-		
Species:	Mouse-A/J - [mouse]-Male		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	2000828		
Domain	Metric	Rating	Comments

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444. Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19) Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s) Rat-Wistar - [rat]-Male Diethylhexyl Phthalate- Parent compound 674255 				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Qu	ality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was identified as di (2-ethylhexyl) phthalate (DEHP). The source and purity (99.9% pure) were reported. Test animal species, strain, sex, age and source were reported. Initial body weight of the animals was not reported. Rats were housed under controlled temperature (exact temperature and humidity not reported) and lighting conditions with a 12 hr day: night cycle. Food and water were available ad libitum. Number of animals/cage were not reported. The concentration levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with qualitative and quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.	
Domain 2: Selection and Performance					
	Metric 2:	Allocation	High	This study is considered High for Metric 2. Allocation of animals to dose groups was done by body weight randomization to ensure equal weight distribution among the groups.	
	Metric 3:	Observational Bias / Blinding Changes	High	This study is considered High for Metric 2.2. Histological examination of testis was per- formed blinded to the treatment group. Other endpoint evaluated were not blinded, but were not subjective in nature (body weight, serum levels of hormones, organ weights, level of mRNA).	
Domain 3. Confounding	/ Variable Con	trol			
	Metric 4:	Confounding / Variable Control	Low	This study is considered Low for Metric 3. The negative control group was appropriate (exposed to air under identical conditions). There is no indication of infection, or any other health condition occurred in the animals. The study does not report the type of cage or water bottle the animals were provided. Co-exposure to plasticizers should be avoided when studying endocrine disruptors such as DEHP because they have the potential to confound the effects of the chemical of interest. It is not clear if steps were made to avoid exposure to plasticis, therefore this metric is rated as low.	
Domain 4: Selective Rer	orting and Attr	ition			
	Metric 5:	Selective Reporting and Attrition	High	This study is considered High for Metric 4. The study does not indicate any animals died. All animals that were exposed were accounted for in the results. Data were provided for all outcomes of interest discussed in the methods.	
Continued on next page					

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Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 674255 Table: 1 of 2

		cont	inued from previ	ous page	
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444. Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19) Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s) Rat-Wistar - [rat]-Male Diethylhexyl Phthalate- Parent compound 674255				
Domain		Metric	Rating	Comments	
Domain 5: Exposure M	fethods Sensiti Metric 6: Metric 7:	vity Chemical administration and characterization Exposure timing, frequency, and duration	Medium High	This study is considered High for Metric 5.1. The purity of the test substance is reported to be 99.9%. A dynamic chamber was used with 15 air changes/hour. DHEP vapor concentration was measured once a day via gas chromatography. The mean concentration with variance were reported as $5.1+/-1.3$ and $24.6 +/-5.2$ mg/m3. Given the variance of >10% of the mean, there is some uncertainty with the exposure. Also, the study does not report at what point in the exposure measurements were made. Only recording one measurement over the six hours invites some uncertainty as to the consistency of exposure throughout the exposure time. This study is considered High for Metric 5.2. In this inhalation study, the route, frequency, and duration of exposure (6 hours/day, 5 days/week, for 4 or 8 weeks) were appropriate for the study type and outcomes of interest.	
Demain (c. Orteane M	[]D.				
	Metric 9:	Endpoint sensitivity and specificity Results presentation	Medium	This study is considered Medium for Metric 6.1. Two exposure groups were studied at two different timepoints. A full range of responses were not obtained; a LOAEL was obtained, but a NOAEL was not. The study did not explain the reasoning for choosing these concentrations. The outcome assessment methodology was adequately reported and cited in HERO 2850042; Lanning et al. 2002. Outcomes were assessed consistently across study groups and appropriate for study interest. This study is considered High for Metric 6.2. Body weight, organ weight, serum hormone levels and mRNA expression are all fully reported with mean, variance and n at both timepoints. Histological findings are reported in text (without incidence data). The proportion of immature tubules is fully reported using grading system. Statistical analysis was appropriate.	
Additional Comments:	None				
Overall Quali	ity Deter	mination	Medium		

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Diethylhexyl	Phthalate
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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444. Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19) Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s) Rat-Wistar - [rat]-Male Diethylhexyl Phthalate- Parent compound 674255			
Domain		Metric	Rating	Comments
Domain 1: Reporting Qu	aality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was identified as di (2-ethylhexyl) phthalate (DEHP). The source and purity (99.9% pure) were reported. Test animal species, strain, sex, age and source were reported. Initial body weight of the animals was not reported. Rats were housed under controlled temperature (exact temperature and humidity not reported) and lighting conditions with a 12 hr day: night cycle. Food and water were available ad libitum. Number of animals/cage were not reported. The concentration levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with qualitative and quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and	l Performance Metric 2:	Allocation	High	This study is considered High for Metric 2. Allocation of animals to dose groups was done by body weight randomization to ensure equal weight distribution among the
	Metric 3:	Observational Bias / Blinding Changes	High	This study is considered High for Metric 2.2. Histological examination of testis was per- formed blinded to the treatment group. Other endpoint evaluated were not blinded, but were not subjective in nature (body weight, serum levels of hormones, organ weights, level of mRNA).

Domain 3: Confounding	/ Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Low	This study is considered Low for Metric 3. The negative control group was appropriate (exposed to air under identical conditions). There is no indication of infection, or any other health condition occurred in the animals. The study does not report the type of cage or water bottle the animals were provided. Co-exposure to plasticizers should be avoided when studying endocrine disruptors such as DEHP because they have the potential to confound the effects of the chemical of interest. It is not clear if steps were made to avoid exposure to plastics, therefore this metric is rated as low.		
Domain 4: Selective Rep	orting and At	trition				
	Metric 5:	Selective Reporting and Attrition	High	This study is considered High for Metric 4. The study does not indicate any animals died. All animals that were exposed were accounted for in the results. Data were provided for all outcomes of interest discussed in the methods.		
Domain 5: Exposure Methods Sensitivity						
Continued on next page						

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674255 Table: 2 of 2

		•••• CO	ntinued from previ	ous page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444. Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19) Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s)			
Exposure Route: Species: Chemical: HERO ID:	Rat-Wistar - [ra Diethylhexyl Ph 674255	t]-Male thalate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 6: 0	Chemical administration and characterization	Medium	This study is considered High for Metric 5.1. The purity of the test substance is reported to be 99.9%. A dynamic chamber was used with 15 air changes/hour. DHEP vapor concentration was measured once a day via gas chromatography. The mean concentration with variance were reported as $5.1+/-1.3$ and $24.6+/-5.2$ mg/m3. Given the variance of >10% of the mean, there is some uncertainty with the exposure. Also, the study does not report at what point in the exposure measurements were made. Only recording one measurement over the six hours invites some uncertainty as to the consistency of exposure throughout the exposure time.

				not report at what point in the exposure measurements were made. Only recording one measurement over the six hours invites some uncertainty as to the consistency of expo- sure throughout the exposure time.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5.2. In this inhalation study, the route, fre- quency, and duration of exposure (6 hours/day, 5 days/week, for 4 or 8 weeks) were appropriate for the study type and outcomes of interest.
Domain 6: Outcome Me	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered Medium for Metric 6.1. Two exposure groups were studied at two different timepoints. A full range of responses were not obtained; a LOAEL was obtained, but a NOAEL was not. The study did not explain the reasoning for choosing these concentrations. The outcome assessment methodology was adequately reported and cited in HERO 2850042; Lanning et al. 2002. Outcomes were assessed consistently across study groups and appropriate for study interest.
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Body weight, organ weight, serum hor- mone levels and mRNA expression are all fully reported with mean, variance and n at both timepoints. Histological findings are reported in text (without incidence data). The proportion of immature tubules is fully reported using grading system. Statistical analy- sis was appropriate.
Additional Comments:	None			

Overall Quality Determination

Medium

May 2025

Study Citation:	Ma, M., Ko	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-				
Health Outcome(s) and Reported Health Effect(c):	Reproductive and uterus of	Reproductive/Developmental-Serum hormones (FSH, LH, testosterone, estradiol); gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary and uterus organ weights, day of vaginal opening-Nutritional/Metabolic-Body weights, food and water intake				
Duration and	Inhalation-V	apor-Duration: Subchronic (>30-90 days)				
Exposure Route:						
Species:	Rat-Other (V	Vistar-Imamichi)-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
	074393					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.		
Domain 3: Confounding	a / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.		
Domain 4: Salaatiya Da	norting and At	trition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.		
Domain 5: Exposure M	ethods Sensitiv	ity				
Continued on next page						

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 1 of 6

Diethylhexyl Phthalate	
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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Ku	urahashi, N., Takeda, M., Kishi	i, R. (2006). Exposure of prepubertal female rats to inhaled di(2-
	ethylhexyl)phthalate affects the onset of puberty a	nd postpubertal reproductive func	ctions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Reproductive/Developmental-Serum hormones (FS	SH, LH, testosterone, estradiol); g	gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary
and Reported	and uterus organ weights, day of vaginal opening-I	Nutritional/Metabolic-Body weig	thts, food and water intake
Health Effect(s):			
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 d	lays)	
Exposure Route:			
Species:	Rat-Other (Wistar-Imamichi)-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	674395		
Demein	M - 4	D-the -	Commente

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The difference between the analytical concentration of the high exposure group (19.78 mg/m3 measured vs. 25 mg/m3 nominal) was ~20%, the range should be within 10% for gases and vapors); no discussion of the difference was provided in the text. The chamber concentrations were measured daily with a gas chromatograph; it was not specified where the air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure device." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., airflow, method of vapor generation) were provided.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6 [,] Outcome Mea	sures and Res	ults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). The authors adequately justified the endpoints assessed and they were sensitive to the outcomes of interest. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were sampled for the endpoints described. The text indicated that animals were sacrifice at PNDs 85-88. The sacrifice time was based on when animals entered the diestrous stage to facilitate measuring serum hormones. It is unclear what effect the different sacrifice times had on other endpoints.

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Study Citation:	Ma, M., Ko	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-				
Health Outcome(s) and Reported Health Effect(s):	ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171. Reproductive/Developmental-Serum hormones (FSH, LH, testosterone, estradiol); gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary and uterus organ weights, day of vaginal opening-Nutritional/Metabolic-Body weights, food and water intake					
Duration and	Inhalation-V	/apor-Duration: Subchronic (>30-90) days)			
Exposure Route:						
Species:	Rat-Other (V	Wistar-Imamichi)-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674395					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	High	Data for all endpoints relevant to these outcomes were presented quantitatively as means \pm SE or SD. The methods of statistical analysis were clearly reported and adequate.		
Additional Comments:	None					
Overall Qualit	y Deterr	nination	Medium			
Study Citation:	Ma, M., Ko	ndo, T., Ban, S., Umemura, T., Kurahash	ni, N., Takeda,	M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-		
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Health Outcome(s) and Reported Health Effect(s): Duration and	envinexy)/phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 95(1):164-171. Reproductive/Developmental-Serum hormones (FSH, LH, testosterone, estradiol); gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary and uterus organ weights, day of vaginal opening-Nutritional/Metabolic-Body weights, food and water intake Inhalation-Vapor-Duration: Subchronic (>30-90 days)					
Exposure Route:						
Species: Chemical: HERO ID:	Rat-Other (W Diethylhexyl 674395	Istar-Imamichi)-Female Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Qu	ality					
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.		
Domain 2: Selection and	Performance					
Domain 2. Selection and	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.		
Domain 3: Confounding	/ Variable Con	ıtrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.		
Domain 1: Selective Per	orting and Att	rition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.		
Domain 5: Exposure Me	thods Sensitivi	ity				
		Contin	ued on next pa	nge		

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 2 of 6

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahash	i, N., Takeda, M., Kishi, R. (2006)	. Exposure of prepubertal female rats to inhaled di(2-
	ethylhexyl)phthalate affects the onset of puberty and post	pubertal reproductive functions. Toxic	ological Sciences 93(1):164-171.
Health Outcome(s)	Reproductive/Developmental-Serum hormones (FSH, LH	l, testosterone, estradiol); gene express	ion in ovaries (real-time RT-PCR), estrous cyclicity, ovary
and Reported	and uterus organ weights, day of vaginal opening-Nutritio	onal/Metabolic-Body weights, food and	l water intake
Health Effect(s):			
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)		
Exposure Route:			
Species:	Rat-Other (Wistar-Imamichi)-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	674395		
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Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The difference between the analytical concentration of the high exposure group (19.78 mg/m3 measured vs. 25 mg/m3 nominal) was ~20%, the range should be within 10% for gases and vapors); no discussion of the difference was provided in the text. The chamber concentrations were measured daily with a gas chromatograph; it was not specified where the air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure device." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., airflow, method of vapor generation) were provided.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome M	leasures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). The authors adequately justified the endpoints assessed and they were sensitive to the outcomes of interest. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were sampled for the endpoints described. The text indicated that animals were sacrifice at PNDs 85-88. The sacrifice time was based on when animals entered the diestrous stage to facilitate measuring serum hormones. It is unclear what effect the different sacrifice times had on other endpoints.

PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
Health Outcome(s)	ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171. Reproductive/Developmental-Serum hormones (FSH, LH, testosterone, estradiol); gene expression in ovaries (real-time RT-PCR), estrous cyclicity, ovary					
and Reported Health Effect(s):	and uterus or	rgan weights, day of vaginal opening	g-Nutritional/Metabolic-B	ody weights, food and water intake		
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)					
Exposure Route: Species:	Rat-Other (Wistar-Imamichi)-Female					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	674395					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	High	Data for all endpoints relevant to these outcomes were presented quantitatively as means \pm SE or SD. The methods of statistical analysis were clearly reported and adequate.		
Additional Comments:	None					
Overall Qualit	y Detern	nination	Medium			

Study Citation:	Ma, M., Ko	ondo, T., Ban, S., Umemura, T., Kurahash	ni, N., Takeda,	M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinic signs)-Undefined clinical signs			
Duration and	Inhalation-V	Vapor-Duration: Subchronic (>30-90 days)		
Exposure Route:	Rat-Other ()	Wistar Imamichi). Female		
Chemical:	Diethylhexy	Phthalate- Parent compound		
HERO ID:	674395			
Domain	1.	Metric	Rating	Comments
Domain 1: Reporting Q	uanty Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection an	d Performance			
Domain 2. Selection an	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding	y / Variable Co	ntrol		
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Re	porting and At	trition		
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other outcomes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure M	ethods Sensitiv	vity		
		Contin	ued on next pa	age

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 3 of 6

Diethylhexyl Phthalate

Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The difference between the analytical concentration of the high exposure group (19.78 mg/m3 measured vs. 25 mg/m3 nominal) was ~20%, the range should be within 10% for gases and vapors); no discussion of the difference was provided in the text. The chamber concentrations were measured daily with a gas chromatograph; it was not specified where the air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure device." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., airflow, method of vapor generation) were provided.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.

Domain 6: Outcome Measures and Results Display

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 3 of 6

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the end- points were assessed after two durations of exposure for comparison. The study au- thors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine- disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thy roid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathol- ogy may not be the most sensitive endpoint to evaluate organ-specific toxicity; how- ever, the purpose of this study was to assess reproductive outcomes, and the absence of histopathology on other organs is not considered to be a study deficiency. The dose con- centrations/spacing were not clearly justified, but the authors stated that the purpose w to evaluate effects upon exposure to "high air doses." All animals were presumably sa pled for the endpoints described, although some data were only qualitatively reported The methods did not mention animal observations, but it was reported in the results the animals showed no signs of toxicity. The text indicated that animals were sacrificed an PNDs 85-88. The sacrifice time was based on when animals entered the diestrous stage to facilitate measuring serum hormones. It is unclear what effect the different sacrifice times had on other endpoints.
	Metric 9:	Results presentation	Medium	Absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs o toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs					
Duration and	Inhalation-V	Vapor-Duration: Subchronic (>30-90 days)				
Exposure Route:						
Species:	Rat-Other (Wistar-Imamichi)-Female				
HERO ID:	674395	1 Phinalate- Parent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality		<u> </u>			
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.		
	15.6					
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.		
Domain 3: Confounding	g / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.		
Domain 4: Selective Pe	norting and At	trition				
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other out-comes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.		
Domain 5: Exposure M	ethods Sensitiv	vity				
		Contin	ued on next pa	age		

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 4 of 6

	continued from previous page
Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The difference between the analytical concentration of the high exposure group (19.78 mg/m3 measured vs. 25 mg/m3 nominal) was ~20%, the range should be within 10% for gases and vapors); no discussion of the difference was provided in the text. The chamber concentrations were measured daily with a gas chromatograph; it was not specified where the air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure device." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., airflow, method of vapor generation) were provided.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
				reproductive functions in remarchais.

Domain 6: Outcome Measures and Results Display

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 4 of 6

... continued from previous page

Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive endpoint to evaluate organ-specific toxicity; however, the purpose of this study was to assess reproductive outcomes, and the absence of histopathology on other organs is not considered to be a study deficiency. The dose con centrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sam pled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results tha animals showed no signs of toxicity. The text indicated that animals were sacrificed at PNDs 85-88. The sacrifice time was based on when animals entered the diestrous stage to facilitate measuring serum hormones. It is unclear what effect the different sacrifice times had on other endpoints.
	Metric 9:	Results presentation	Medium	Absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-					
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs					
Duration and	Inhalation-V	/apor-Duration: Subchronic (>30-90 days)				
Exposure Route:						
Species:	Rat-Other (Wistar-Imamichi)-Female				
HERO ID:	674395	r Finnanae- Farent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.		
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.		
Domain 3: Confounding	g / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.		
Domain 4: Selective Re	porting and At	trition				
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other out-comes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.		
Domain 5: Exposure M	ethods Sensitiv	vity				
		Contin	ued on next pa	age		

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 5 of 6

Diethylhexyl Phthalate	Human Hea

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
Metric 6	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The difference between the analytical concentration of the high exposure group (19.78 mg/m3 measured vs. 25 mg/m3 nominal) was ~20%, the range should be within 10% for gases and vapors); no discussion of the difference was provided in the text. The chamber concentrations were measured daily with a gas chromatograph; it was not specified where the air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure device." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., airflow, method of vapor generation) were provided.
Metric 7	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.

Domain 6: Outcome Measures and Results Display

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 5 of 6

Diethylhexyl Phthalate

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive endpoint to evaluate organ-specific toxicity; however, the purpose of this study was to assess reproductive outcomes, and the absence o histopathology on other organs is not considered to be a study deficiency. The dose co centrations/spacing were not clearly justified, but the authors stated that the purpose were effects upon exposure to "high air doses." All animals were presumably sa pled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results th animals showed no signs of toxicity. The text indicated that animals were sacrificed at PNDs 85-88. The sacrifice time was based on when animals entered the diestrous stag to facilitate measuring serum hormones. It is unclear what effect the different sacrifice times had on other endpoints.
	Metric 9:	Results presentation	Medium	Absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs o toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2- ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171. Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs			
Health Outcome(s) and Reported Health Effect(s):				
Duration and	Inhalation-V	/apor-Duration: Subchronic (>30-90 days)		
Exposure Route:				
Species:	Rat-Other (Wistar-Imamichi)-Female		
Chemical:	Diethylhexy	Phthalate- Parent compound		
HERO ID:	674395			
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were
	Metric 3:	Observational Bias / Blinding Changes	Medium	described. Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 2: Confoundin	a / Variabla Ca	ntuol		
Domain 3: Contounding	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Re	porting and At	trition		
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other out-comes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure M	ethods Sensitiv	vity		
		Contin	ued on next pa	

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 674395 Table: 6 of 6

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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.			
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical			
and Reported	signs)-Undefined clinical signs			
Health Effect(s):				
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)			
Exposure Route:				
Species:	Rat-Other (Wistar-Imamichi)-Female			
Chemical:	Diethylhexyl Phthalate- Parent compound			
HERO ID:	674395			

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The difference between the analytical concentration of the high exposure group (19.78 mg/m3 measured vs. 25 mg/m3 nominal) was ~20%, the range should be within 10% for gases and vapors); no discussion of the difference was provided in the text. The chamber concentrations were measured daily with a gas chromatograph; it was not specified where the air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposure swere whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure device." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., airflow, method of vapor generation) were provided.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.

Domain 6: Outcome Measures and Results Display

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HERO ID: 674395 Table: 6 of 6

Diethylhexyl P	hthalate
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Study Citation:	Ma, M., Kondo, T., Ban, S., Umemura, T., Kurahashi, N., Takeda, M., Kishi, R. (2006). Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicological Sciences 93(1):164-171.
Health Outcome(s)	Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical
and Reported	signs)-Undefined clinical signs
Health Effect(s):	
Duration and	Inhalation-Vapor-Duration: Subchronic (>30-90 days)
Exposure Route:	
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). Organ weights in the absence of histopathology may not be the most sensitive endpoint to evaluate organ-specific toxicity; however, the purpose of this study was to assess reproductive outcomes, and the absence of histopathology on other organs is not considered to be a study deficiency. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were presumably sampled for the endpoints described, although some data were only qualitatively reported. The methods did not mention animal observations, but it was reported in the results that animals showed no signs of toxicity. The text indicated that animals were sacrificed at PNDs 85-88. The sacrifice time was based on when animals entered the diestrous stage to facilitate measuring serum hormones. It is unclear what effect the different sacrifice times and other endpoints.
	Metric 9:	Results presentation	Medium	Absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Akingbemi,	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple					
Health Outcome(s) and Reported Health Effect(s):	endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780. Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stareology); aromatese gape expression in Leydig cells.						
Duration and	Oral-Gavage	e-Duration: Chronic (>90 days)-7-100-day(s)				
Exposure Route:	c						
Species:	Rat-Long-E	vans - [rat]-Male					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	673552						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Low	The study does not report how animals were allocated to study groups. No other meth- ods to control for modifying factors across groups were noted.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).			
Domain 3: Confounding	a / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Food and water dispensing containers were not described.			
Domain 4: Selective Re	porting and At	trition					
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for most prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.			
Domain 5: Exposure M	ethods Sensitiv	vity					
		Contin	ued on next pa	ıge			

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673552 Table: 1 of 2

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Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple
Health Outcome(s)	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and astradial form isolated Laudia cella (head and after LU strainality) Laudia cella realification (assessed by 1) and a straticity of the second strainality

Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780. Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s) Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound						
HERO ID:	673552						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.			
Domain 6: Outcome Me	asures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.			
	Metric 9:	Results presentation	Medium	Results for most endpoints were described in the text and data were presented in tables as means \pm standard error. Statistical tests were reported and appropriate. Histopathological data were reported as negative in the text.			
Additional Comments:	Study includ performed of evaluation of	ded 3 different durations of exposure during on different cohorts of animals (though thi corresponds with the PND21-120 duration	post weaning up s is unclear and a	to adulthood. each "experiment" included different outcomes and were potentially all groups were >10). not all outcomes were evaluated for each duration. This			

Overall Quality I	Determination
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Medium

Study Citation:	Akingbemi, endocrine di	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.						
Health Outcome(s) and Reported Health Effect(s):	Nutritional/I	Nutritional/Metabolic-Body weight Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s)						
Duration and	Oral-Gavage							
Exposure Route: Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673552	vans - [rat]-Male l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.				
Domain 2: Selection an	d Performance							
	Metric 2:	Allocation	Low	The study does not report how animals were allocated to study groups. No other meth- ods to control for modifying factors across groups were noted.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).				
Domain 3: Confounding	g / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive con- trol group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates. Food and water dispensing containers were not described. However, the missing information is not expected to significantly impact the endpoints described.				
Domain 4: Selective Re	porting and At	trition						
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for all prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.				
Domain 5: Exposure M	ethods Sensitiv	rity						
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.				
		Continu	ued on next pa					

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Diethylhexyl Phthalate

		conti	nued from previ	ous page				
Study Citation: Health Outcome(s) and Reported	Akingbemi, endocrine di Nutritional/I	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780. Nutritional/Metabolic-Body weight						
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s)						
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 673552	Rat-Long-Evans - [rat]-Male Diethylhexyl Phthalate- Parent compound 673552						
Domain		Metric	Rating	Comments				
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.				
Domain 6: Outcome Me	easures and Re	sults Display						
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough (comparable to recommended by guidance, 10) and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.				
	Metric 9:	Results presentation	High	Results were reported as means \pm standard error. Statistical tests were reported and appropriate.				
Additional Comments:	Study included 3 different durations of exposure during post weaning up to adulthood. each "experiment" included different outcomes and were potentially performed on different cohorts of animals (though this is unclear and all groups were >10). not all outcomes were evaluated for each duration. This evaluation corresponds with the PND21-120 duration							
Overall Qualit	ty Deteri	nination	Medium					

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Study Citation:	Ganning, A.	E., Olsson, M. J., Brunk, U., Dallner, G. (19	990). Effect	s of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology			
Health Outcome(s) and Reported Health Effect(s):	67(5):392-401. Nutritional/Metabolic-Body Weight						
Duration and	Oral-Diet-D	uration: Chronic (>90 days)-7-24-102-weel	k(s)				
Species:	Rat-Sprague	e-Dawley - [rat]-Male					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
	679540						
Domain Domain 1: Reporting Or	uality	Metric	Rating	Comments			
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.			
Domain 4: Selective Reporting and Attrition							
Continued on next page							

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		cont	tinued from p	previous page				
Study Citation:	Ganning, A 67(5):392-4	. E., Olsson, M. J., Brunk, U., Dallner, G. 01.	(1990). Effect	s of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology				
Health Outcome(s) and Reported	Nutritional/	Metabolic-Body Weight						
Health Effect(s):								
Exposure Route:	Orai-Diet-D	Juration: Chronic (>90 days)-7-24-102-w	eek(s)					
Species:	Rat-Sprague	e-Dawley - [rat]-Male						
Chemical:	Diethylhexy	l Phthalate- Parent compound						
HERO ID:	679540							
Domain		Metric	Rating	Comments				
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.				
Domain 5: Exposure Mo	ethods Sensitiv	vity						
ľ	Metric 6:	Chemical administration and characterization	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.				
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).				
Domain 6: Outcome Me	easures and Re	esults Display						
	Metric 8:	Endpoint sensitivity and specificity	Medium	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. The outcome assessment methodology (measuring body weight throughout the study) addressed the outcome of interest and from the information we have, it appears that it was applied con- sistently. The sampling was adequate for the intended outcome.				
	Metric 9:	Results presentation	Low	Body weight data was presented in a figure presumably showing means with no mea- sures of variance. Statistical analysis was performed but statistical methods were not adequately described. Individual animal data were not provided.				
Additional Comments:	None							
<u> </u>		•	Ŧ					
Overall Qualit	ty Deteri	mination	Low					

Study Citation:	Ganning, A.	E., Olsson, M. J., Brunk, U., Dallner, G. (1990)	. Effects of prolonge	d treatment with phthalate ester on rat liver. Pharmacology & Toxicology				
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testes histology and cell appearance; testes function as measured by inhibition of spermatogenesis and instances of general tubular atrophy							
Duration and Exposure Route:	Oral-Diet-Du	uration: Chronic (>90 days)-7-24-102-week(s)						
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexyl 679540	Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound 679540						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.				
Domain 2: Selection an	d Performance							
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.				
Domain 3: Confounding	g / Variable Cor	ntrol						
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of the test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. Insufficient information has been provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard. The study did not specify whether measures were taken to reduce exposure to other plasticizers, and this may have a significant impact on endpoints affected by endocrine disruption.				
Domain 4: Selective Re	porting and Att	rition						

Human Health Hazard Animal Toxicology Evaluation

			continued from previous	page			
Study Citation:	Ganning, A. 67(5):392-4	E., Olsson, M. J., Brunk, U., Dallner, G. (1) 01.	990). Effects of prolonged	l treatment with phthalate ester on rat liver. Pharmacology & Toxicology			
Health Outcome(s)	Reproductive/Developmental-Testes histology and cell appearance; testes function as measured by inhibition of spermatogenesis and instances of general						
and Reported	tubular atrophy						
Health Effect(s):							
Duration and	Oral-Diet-D	uration: Chronic (>90 days)-7-24-102-wee	k(s)				
Exposure Koute:	Dat Sprague	Dowlay [rot] Male					
Chemical:	Diethylhexy	Phthalate- Parent compound					
HERO ID:	679540						
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.			
Domain 5: Exposure M	ethods Sensitiv	vity					
Domain 5. Exposure M	Metric 6:	Chemical administration and	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate			
		characterization		of analysis was included but likely was available from the supplier at the time of pur- chase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were pro- vided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).			
Domain 6: Outcome M	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. The outcome assessment methods for conducting histopathology were not reported. Other endpoints to assess the toxicity of the target organ (e.g., organ weights) were not evaluated. The sample sizes were not specified for histopathology and the number of animals per group is unclear. Animal source and age were not reported.			
	Metric 9:	Results presentation	Uninformative	Exposure-related effects were qualitatively described in the text (testis histology). Sta- tistical methods were not described, and the statistical significance of the changes is unknown. No data are available for an independent analysis.			
Additional Comments:	None						

Overall Quality Determination

Uninformative

Study Citation:	Ganning, A. 1 67(5):392-40 Canaar/Carai	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.					
Health Outcome(s)and ReportedHealth Effect(s):Duration andExposure Route:Species:Chemical:HERO ID:	Cancer/Carcinogenesis-Tumors-Other (please specify below) ("general health")-Assessment of "general health" Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s) Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality	monie	Ruting	connicito			
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.			
Domain 2: Selection and	d Performance						
Domain 2. Selection and	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.			
Domain 3: Confounding	r / Variable Con	trol					
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.			
Domain 4: Selective Re	norting and Att	rition					
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.			
		Conti	nued on nex	at page			

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 679540 Table: 3 of 6

		cont	tinued from p	previous page			
Study Citation:	Ganning, A 67(5):392-4	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.					
Health Outcome(s) and Reported Health Effect(s):	Cancer/Carcinogenesis-Tumors-Other (please specify below) ("general health")-Assessment of "general health"						
Duration and Exposure Route:	Oral-Diet-D	Puration: Chronic (>90 days)-7-24-102-we	eek(s)				
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 679540	e-Dawley - [rat]-Male I Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 5: Exposure M	ethods Sensitiv Metric 6: Metric 7:	vity Chemical administration and characterization Exposure timing, frequency, and duration	Low High	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values. The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).			
Domain 6: Outcome M	easures and Re Metric 8: Metric 9:	sults Display Endpoint sensitivity and specificity Results presentation	Low Medium	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme func- tion). The authors clearly justified the use of low exposure levels. The outcome assess- ment methodologies for clinical observations were missing and details of histological examinations were insufficient. Sample sizes were not specified for either outcome and the number of animals per group is unclear. Animal source and age were not reported. Animals were qualitatively reported to be "in good health" throughout the study. The text was suggestive that no treatment-related tumors were observed, but no data were provided.			
Additional Comments:	None						
Overall Quali	ty Deteri	mination	Low				

Health Outcome(s)	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401. Cancer/Carcinogenesis-Tumors-Other (please specify below) ("general health")-Assessment of "general health" Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s) Rat-Sprague-Dawley - [rat]-Male Diethylhexyl Phthalate- Parent compound 679540					
and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:						
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.		
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were relected for the various times of secrifice		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.		
Domain 2: Confounding	y / Variabla Con	trol				
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.		
Domain 4: Selective Re	porting and Att	rition				
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.		

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 679540 Table: 4 of 6

		cont	tinued from p	previous page			
Study Citation:	Ganning, A 67(5):392-4	. E., Olsson, M. J., Brunk, U., Dallner, G. 01.	(1990). Effect	s of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology			
Health Outcome(s) and Reported Health Effect(s):	Cancer/Carcinogenesis-Tumors-Other (please specify below) ("general health")-Assessment of "general health"						
Duration and Exposure Route:	Oral-Diet-D	Ouration: Chronic (>90 days)-7-24-102-we	eek(s)				
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 679540	e-Dawley - [rat]-Male /l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 5: Exposure M	ethods Sensitiv Metric 6:	vity Chemical administration and	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate			
		characterization		of analysis was included but likely was available from the supplier at the time of pur- chase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were pro- vided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Low	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme func- tion). The authors clearly justified the use of low exposure levels. The outcome assess- ment methodologies for clinical observations were missing and details of histological examinations were insufficient. Sample sizes were not specified for either outcome and the number of animals per group is unclear. Animal source and age were not reported.			
	Metric 9:	Results presentation	Medium	Animals were qualitatively reported to be "in good health" throughout the study. The text was suggestive that no treatment-related tumors were observed, but no data were provided.			
Additional Comments:	None						
Overall Quali	ty Deteri	mination	Low				

Study Citation:	Ganning, A.	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology						
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl- transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)							
Duration and	Oral-Diet-Du	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)						
Species:	Rat-Sprague-	-Dawley - [rat]-Male						
Chemical:	Diethylhexyl	Phthalate- Parent compound						
Demoin	079340	Matria	Dating	Comments				
Domain 1: Reporting O	uality	Metric	Katilig	Comments				
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.				
Domain 2: Selection and	d Performance	A11 - 2	т					
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.				
Domain 3: Confounding	y / Variable Cor	ntrol						
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed normal diets. Positive con- trols are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.				
Domain 4: Selective Rep	porting and Att	rition						
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.				
		Conti	nued on nex	at page				

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Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 679540 Table: 5 of 6

		cont	inued from J	previous page			
Study Citation:	Ganning, A	. E., Olsson, M. J., Brunk, U., Dallner, G. ((1990). Effec	ts of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicolog			
Health Outcome(s) and Reported Health Effect(s):	67(5):392-401. Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)						
Duration and Exposure Route:	Oral-Diet-D	Duration: Chronic (>90 days)-7-24-102-we	eek(s)				
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 679540	e-Dawley - [rat]-Male yl Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
Domain 5: Exposure M	ethods Sensitiv Metric 6:	vity Chemical administration and	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate			
		characterization		of analysis was included but likely was available from the supplier at the time of pur- chase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were pro- vided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Low	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. Animal source and age were not reported. Methodological details for conducting electron microscopy were adequately described. Methods for isolation of hepatic fractions and enzyme measurements were cited to other studies that were not available for review. Protein determination was done according to Lowry et al. (1951), which is a known and accepted method. Sample sizes ranged from 9-14 or from 6-11 for most endpoints. No details on sampling were provided. Histopathology details were insufficiently described.			
	Metric 9:	Results presentation	Low	Results for liver-related endpoints were not adequately reported. Representative images of electron microscopy findings were provided along with detailed descriptions in the text. These data were not statistically analyzed. Protein and enzyme activity data was presented in figures presumably reporting mean values with no measures of variance. Additional "statistical evaluation" tables were included to specify statistical significance, but the statistical methods used were not described, and individual animal data were not available to conduct an independent analysis. The lack of histopathology findings was stated in the study text.			
Additional Comments:	None						
Overall Quali	ty Deteri	mination	Low				

Study Citation:	Ganning, A.	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401					
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Live transferase a	er-Liver histology, (both light and electron mic and cytochrome oxidase (mitochondria), and C	croscopy); liver enzyme YP-450, NADH and NA	e activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl- ADPH cytochrome c reductase (microsomes)			
Duration and	Oral-Diet-D	uration: Chronic (>90 days)-7-1-year(s)					
Exposure Koute: Species:	Rat-Sprague	-Dawley - [rat]-Male					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	679540						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality		Madiana				
	Metric 1:	Reporting Quality	Medium	Critical information (SD rats, test article identified by name, dose level, duration of ex- posure, exposure route, and qualitative or quantitative results for at least one endpoint) was reported; however, no additional details were provided. Neither the number of ani- mals per group or the sample size was specified.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group fed diets in the absence of test substance; however, it does not appear that control animals were included during the post-exposure period. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study.			
Domain 4: Selective Pe	porting and At	trition					
Domain 4. Selective Re	Metric 5:	Selective Reporting and Attrition	Low	Insufficient information was provided to determined attrition or selective reporting.			
Domain 5: Exposure M	ethods Sensitiv	vitv.					
	Metric 6:	Chemical administration and characterization	Uninformative	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Neither feed intake nor body weights were measured and reliable doses in mg/kg-day cannot be determined.			
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure timing and frequency were reported. Animals were exposed via the diet for 1 year. No justification was provided by the study author.			
Domain 6: Outcome Me	easures and Re	sults Display					

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 679540 Table: 6 of 6

		c	ontinued from previous	page				
Study Citation:	Ganning, A.	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):302.401						
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liv transferase	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl- transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)						
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-1-year(s)							
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 679540	e-Dawley - [rat]-Male l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	The study included a single endpoint (measurement of hepatic enzyme activities). It is unclear what the purpose of the study was. Enzyme activities alone are not considered to be a sensitive endpoint for assessing hepatic toxicity. No methodological details were provided, although similar measurements were reported for another experiment reported in the same study. Methods of enzyme measurements were cited to other sources.				
	Metric 9:	Results presentation	Uninformative	Data were reported in figures showing means without measures of variance for the con- trol and high-dose groups only. Results for the 0.2% group were briefly described in the study text. It does not appear that statistical analysis was conducted and insufficient in- formation was provided to conduct an independent analysis.				
Additional Comments:	None							
	_							

Overall Quality Determination

Uninformative

Study Citation:	Laws, M. J.,	Meling, D. D., Deviney, K., A.R., Santacruz	-Márquez, R., I	Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl			
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake						
Duration and	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)						
Exposure Route:							
Species:	Mouse-CD-1	- [mouse]-Female					
HERO ID:	11784622	Thualace Tarent compound					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	Medium	The test material, source and purity were reported. Other reported information included details on the test model (species, strain, source, and age), number of animals/per cage, experimental design, number of animals per group, endpoint evaluation methods, and results for the endpoint of interest. Food was provided ad libitum. Missing information included animal husbandry (water availability, temperature, humidity, light cycle) and initial body weights. Although husbandry conditions were not reported, the study does state "The University of Illinois Institution Animal Care and Use Committee approved all animal handling, housing, and procedure", therefore it can be reasonably assumed animals were maintained in a humane and scientifically sound manner.			
Domain 2: Selection an	d Derformance						
Domain 2. Selection and	Metric 2:	Allocation	Medium	The animals were randomly allocated to study groups, but the method of allocation was not further described.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Investigators were unblinded to mouse identification for assessment of body weight, food intake, and fertility indices; however, these endpoints were quantitative, and lack of blinding is unlikely to substantially impact results. Cytologist were blinded when assessing estrous cyclicity.			
Domain 3: Confounding	a / Variable Cor	atrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included. Low levels of phthalate metabolites were de- tected in the urine of control mice. Authors state it is difficult to completely eliminate phthalate exposure and speculate low levels of phthalate may have been in the drink- ing water (reverse osmosis water provided) or may have leached into the urine samples from plastic tubing used. No positive control was included nor required for the study. Consistency of other potentially confounding factors (body weight and food intake) was reported. Mice were housed in polysulfone cages with 1/8 corn cob bedding. Drink- ing water was purified by reverse osmosis. It was not explicitly specified whether food, water, or bedding was tested for contaminates, but speciality food mixtures and reverse osmosis were used indicating some attention was made to try to minimize unwanted ex- posures. The materials used to dispense water to the animals were not specified. Most husbandry conditions were not reported, these missing details are not expected to have a significant impact on the study results. Study groups were evaluated under comparable conditions.			

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Diethylhexyl Phthalate

		cont	inued from previ	ous page			
Study Citation:	Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl						
Health Outcome(s) and Reported Health Effect(s):	phthalate, and a mixture of phthalates alters estrous cyclicity and/or impairs gestational index and birth rate in mice. Toxicological Sciences 193(1):48-61. Nutritional/Metabolic-Body weight and food intake						
Duration and	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)						
Exposure Route:			5)				
Species:	Mouse-CD-	1 - [mouse]-Female					
Chemical:	Diethylhexy	Phthalate- Parent compound					
HERO ID:	11784622						
Domain		Metric	Rating	Comments			
Domain 4: Selective R	eporting and At	trition					
	Metric 5:	Selective Reporting and Attrition	Low	Twelve to fourteen mice began the study and were assessed for body weight, food intake and estrous cycle for 11 months. Urinary metabolites were assessed in 4-8 mice, and only 7-9 females were used for the breeding portion of the study. The study authors did not report how they chose the animals or why only select animals were used.			
Domain 5: Exposure M	lethods Sensitiv	vity					
Domain 5: Exposure W	Metric 6:	Chemical administration and characterization Exposure timing, frequency, and duration	Low	The test material source (Sigma-Aldrich) was reported. The purity was reported to be \geq 98%. No certificate of analysis was provided in the study report and there is no indication that the test substance was verified by the performing laboratory. Details on the preparation are limited. The test substances was mixed in corn oil and provided to Envigo Teklad Diets (Madison, WI) for chow preparation, which was then delivered to the authors. The concentration of the test material in the food was not verified. Storage information was not reported, and it is in unclear if one formulation was used for the entire 11 months of exposure. Although study authors measured body weight and food intake, they did not use these measurements to calculate daily intake, rather they based their calculation of dose on the assumption that a 25-gram mouse eats approximately 5 grams of food/day. Therefore, only a target dose in mg/kg-day was provided. Body weights are reported as a change in body weight (initial not reported), therefore not enough information is provided to calculate the dose independently. Urinary metabolites were measured and increased with increased concentration, therefore there is evidence animals were receiving phthalates in their diet. The exposure timing, frequency and duration were appropriate for outcomes of interest. Reported information indicates exposure was consistent with timing and frequency			
Domain 6: Outcome M	easures and De	sulte Diculay		across study groups. However, there is a discrepancy in the study report. The methods specify exposure for 11 months, but figure legends for Figures 6-8 specify 12 months.			
Domain 0. Outcome M	Metric 8:	Endpoint sensitivity and specificity	High	The species was appropriate to evaluate outcomes of interest. The number of females used to assess body weights and food intake was sufficient. A wide range of concentrations (0.15-1500 ppm in food) were studied. Outcomes were assessed consistently across study groups. There are no major concerns regarding the outcome methodology for outcomes of interest.			
	Metric 9:	Results presentation	Medium	Body weight and food intake were fully reported as means +/- SEM for the first 11 months of exposure. Body weights during breeding and gestation were not reported. Statistical analysis was performed and appropriate.			

Continued on next page ...

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl phthalate, and a mixture of phthalates alters estrous cyclicity and/or impairs gestational index and birth rate in mice. Toxicological Sciences 193(1):48-61. Health Outcome(s) Nutritional/Metabolic-Body weight and food intake and Reported Health Effect(s): **Duration and** Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s) **Exposure Route:** Species: Mouse-CD-1 - [mouse]-Female Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 11784622 Domain Metric Rating Comments Additional Comments: None

Overall Quality Determination

Medium

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl phthalate, and a mixture of phthalates alters estrous cyclicity and/or impairs gestational index and birth rate in mice. Toxicological Sciences 193(1):48-61. Reproductive/Developmental-Estrous cycle; Fertility indices (mating index, gestational index, pregnancy, birth rate, dystocia and fertility index) Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s) Mouse-CD-1 - [mouse]-Female Diethylhexyl Phthalate- Parent compound 1178/622					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test material, source and purity were reported. Other reported information included details on the test model (species, strain, source, and age), number of animals/ per cage, experimental design, number of animals per group, endpoint evaluation methods, and results for the endpoint of interest. Food was provided ad libitum. Missing information included animal husbandry (water availability, temperature, humidity, light cycle) and initial body weights. Although husbandry conditions were not reported, the study does state "The University of Illinois Institution Animal Care and Use Committee approved all animal handling, housing, and procedure", therefore it can be reasonably assumed animals were maintained in a humane and scientifically sound manner.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	Medium	The animals were randomly allocated to study groups, but the method of allocation was not further described.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Investigators were unblinded to mouse identification for assessment of body weight, food intake, and fertility indices; however, these endpoints were quantitative, and lack of blinding is unlikely to substantially impact results. Cytologist were blinded when assessing estrous cyclicity.		
Domain 3: Confounding	y / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included. Low levels of phthalate metabolites were de- tected in the urine of control mice. Authors state it is difficult to completely eliminate phthalate exposure and speculate low levels of phthalate may have been in the drink- ing water (reverse osmosis water provided) or may have leached into the urine samples from plastic tubing used. No positive control was included nor required for the study. Consistency of other potentially confounding factors (body weight and food intake) was reported. Mice were housed in polysulfone cages with 1/8 corn cob bedding. Drink- ing water was purified by reverse osmosis. It was not explicitly specified whether food, water, or bedding was tested for contaminates, but speciality food mixtures and reverse osmosis were used indicating some attention was made to try to minimize unwanted ex- posures. The materials used to dispense water to the animals were not specified. Most husbandry conditions were not reported, these missing details are not expected to have a significant impact on the study results. Study groups were evaluated under comparable conditions.		

Domain 4: Selective Reporting and Attrition

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 11784622 Table: 2 of 2

		con	tinued from p	revious page			
Study Citation: Health Outcome(s) and Reported	Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl phthalate, and a mixture of phthalates alters estrous cyclicity and/or impairs gestational index and birth rate in mice. Toxicological Sciences 193(1):48-61. Reproductive/Developmental-Estrous cycle; Fertility indices (mating index, gestational index, pregnancy, birth rate, dystocia and fertility index)						
Health Effect(s): Duration and	Oral-Diet-D	uration: Chronic (>90 days)-7-11-month	(s)				
Exposure Koute: Species:	Mouse-CD-	1 - [mouse] Female					
Chemical: HERO ID:	Diethylhexy 11784622	l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Low	Twelve to fourteen mice began the study and were assessed for body weight, food intake and estrous cycle for 11 months. Urinary metabolites were assessed in 4-8 mice, and only 7-9 females were used for the breeding portion of the study. The study authors did not report how they chose the animals or why only select animals were used.			
Domain 5: Exposure M	ethods Sensitiv	vity					
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma-Aldrich) was reported. The purity was reported to be \geq 98%. No certificate of analysis was provided in the study report and there is no indication that the test substance was verified by the performing laboratory. Details on the preparation are limited. The test substances was mixed in corn oil and provided to Envigo Teklad Diets (Madison, WI) for chow preparation, which was then delivered to the authors. The concentration of the test material in the food was not verified. Storage information was not reported, and it is in unclear if one formulation was used for the entire 11 months of exposure. Although study authors measured body weight and food intake, they did not use these measurements to calculate daily intake, rather they based their calculation of dose on the assumption that a 25-gram mouse eats approximately 5 grams of food/day. Therefore, only a target dose in mg/kg-day was provided. Body weights are reported as a change in body weight (initial not reported), therefore not enough information is provided to calculate the dose independently. Urinary metabolites were measured and increased with increased concentration, therefore there is evidence animals were receiving phthalates in their diet.			
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure timing, frequency and duration were appropriate for outcomes of interest. Reported information indicates exposure was consistent with timing and frequency across study groups. However, there is a discrepancy in the study report. The methods specify exposure for 11 months, but figure legends for Figures 6-8 specify 12 months.			
Domain 6: Outcome M	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	The species was appropriate to evaluate outcomes of interest. The number of females used to assess fertility indices (n=6-9) is less than recommended in OECD 421 guide- lines (n=12-13). A wide range of concentrations (0.15-1500 ppm in food) were studied. Outcomes were assessed consistently across study groups. There are no major concerns regarding the outcome methodology for outcomes of interest.			
		Cor	ntinued on nex	xt page			
Human Health Hazard Animal Toxicology Evaluation

HERO ID: 11784622 Table: 2 of 2

			continued from p	previous page		
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., Flaws, J. A. (2023). Long-term exposure to di(2-ethylhexyl) phthalate, diisononyl phthalate, and a mixture of phthalates alters estrous cyclicity and/or impairs gestational index and birth rate in mice. Toxicological Sciences 193(1):48-61. Reproductive/Developmental-Estrous cycle; Fertility indices (mating index, gestational index, pregnancy, birth rate, dystocia and fertility index)					
Duration and Exposure Route:	Oral-Diet-D	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)				
Species: Chemical: HERO ID:	Mouse-CD- Diethylhexy 11784622	1 - [mouse]-Female 1 Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	Low	Estrous cycle data were fully reported as means +/- SEM along with individual animal data (graphically). Fertility indices were reported as percentages. Statistical analysis was performed and was appropriate. Data for several endpoints were recorded to facilitate the calculation of reproductive indices (e.g., birth index, gestation index etc.,), but the data were not reported. Some of these endpoints include the gestation length, number of pregnant dams, number of live pups, and number of dams that gave birth . The lack of these data precludes the ability to, for example, conduct a trend test for the gestation index that has a borderline significant value at the mid-dose, which would help with the interpretation of the study results. Exclusion of the data for these endpoints in study types that report reproductive indices is atypical.		
Additional Comments:	None					
Overall Ouali	tv Deteri	nination	Low			

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Santacruz-M effects of sho 110(1):198-2 Reproductive tissue-Other Oral-Diet-Du Mouse-CD-1 Diethylhexyl 11784618	Santacruz-Márquez, R., Safar, A. M., Laws, M. J., Meling, D. D., Liu, Z., Kumar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The effects of short-term and long-term phthalate exposures on ovarian follicle growth dynamics and hormone levels in female mice [†] . Biology of Reproduction 110(1):198-210. Reproductive/Developmental-Ovary histopathology, serum hormones (progesterone, testosterone, estradiol, FSH, LH), and gene expression in ovarian tissue-Other (please specify below) (Endocrine)-Gene expression in pituitary tissue Oral-Diet-Duration: Chronic (>90 days)-7-6-month(s) Mouse-CD-1 - [mouse]-Female Diethylhexyl Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified as di(2-ethylhexyl)phthalate. No CASRN was pro- vided. The test substance was sourced from Sigma-Aldrich (St. Louis, MO). Test animal species, strain, sex, age, and source were reported. It was not specified whether mice were virgins (33 days old at purchase), and Initial body weights were not reported. Hus- bandry conditions (temperature, humidity, and light cycle) were not reported. Animals were housed 3/cage. Feed and water were available ad libitum. Dose levels (ppm), du- ration, and route of exposure were reported; however, the number of animals/group was not clearly stated, but sample sizes for each endpoint were specified. Target concentra- tions were reported; however, actual doses were not. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Low	Allocation methods were not reported.	
	Metric 3:	Observational Bias / Blinding Changes	High	Humans that were counting the follicle populations were blinded to treatments. Blinding for other measures was not reported; however, the endpoints evaluated were either not subjective in nature or consisted of histopathology.	
Domain 3: Confounding	g / Variable Con Metric 4:	ttrol Confounding / Variable Control	Low	Body weight and food intake were not reported in a study with dietary exposures. The authors cited a previous study by the same group that showed exposure to the test sub- stance via the diet did not affect body weight or food consumption. A negative control group was included (rodent chow with 7% corn oil) and responses were appropriate for negative controls. Housing conditions (e.g., bedding, RO water, animals per cage) were consistent across groups but animal husbandry details (temperature, humidity etc.,) were not reported. The study did not indicate whether measures were taken to reduce exposure to plasticizers from bedding, feed, or equipment (e.g., water dispensers). No testing for contaminates was described and the study was assessing endocrine disruption. The study noted that animals were sacrificed in diestrus. No further details were provided and it is unclear whether sacrifices were conducted on the same day.	

Domain 4: Selective Reporting and Attrition

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 11784618 Table: 1 of 1

		conti	inued from previ	ious page			
Study Citation:	Santacruz-M effects of sh 110(1):198-	Santacruz-Márquez, R., Safar, A. M., Laws, M. J., Meling, D. D., Liu, Z., Kumar, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The effects of short-term and long-term phthalate exposures on ovarian follicle growth dynamics and hormone levels in female mice [†] . Biology of Reproduction 110(1):198-210. Reproductive/Developmental-Ovary histopathology, serum hormones (progesterone, testosterone, estradiol, FSH, LH), and gene expression in ovarian					
Health Outcome(s)	Reproductiv						
and Reported	tissue-Other (please specify below) (Endocrine)-Gene expression in pituitary tissue						
Health Effect(s):		(f)) () ()					
Duration and	Oral-Diet-F	Duration: Chronic (>90 days)-7-6-month(s)					
Exposure Route	Ofui Diet D	variation: enronne (> >0 days) + 0 month(s)					
Spacios	Mouse-CD-	1 - [mouse]-Female					
Chomical:	Diethylbey	I Phthalate Darent compound					
	11704610	a ratent compound					
HERO ID:	11/84018						
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for almost all outcomes. The methods stated that pituitary glands were collected for analysis of pituitary gene expression. It is unclear from the text whether pituitaries were collected from both short-term and chronic duration experiments, but results were only reported for the long-term exposure groups. Insufficient information was provided to assess attrition. The number of animals per group was not specified in the methods and sample sizes varied from 3-8 per endpoint and in some cases, numbers varied from 4-6 within an endpoint. No justification for the differences in sample sizes was provided and it is unclear if this represents selective reporting.			
Domain 5: Exposure M	Methods Sensitiv Metric 6: Metric 7:	vity Chemical administration and characterization Exposure timing, frequency, and duration	Low	The purity of the test substance was not reported; however, the Ssource was specified (Sigma-Aldrich, and purities on the supplier website were all >98%. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. Envigo Tekland was supplied with the test substance in corn oil, and the diets were prepared (no additional details were provided). Target test concentrations in food (ppm) were reported; there is no indication that analysis was done. The authors provided "rough equivalents" in mg/kg-day; however, it was not specified how these estimates were made - Only target concentrations were reported; no analysis was done. No feed intake or body weights were recorded and ADD was not calculated. Dietary exposure was selected to mimic human exposure. The timing, duration, and frequency were appropriate for the study type and the outcomes of interest. The durations were justified by the study authors.			
Domain 6: Outcome M	Aeasures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. A limited number of endpoints were assessed but were in line with the specified goals of the study. Outcome methodologies were reported and were sensitive to the outcomes of interest. The test animal species was appropriate and obtained from a commercial source. The exposure concentrations were based on a previously published rationale and were meant to fall within daily human exposure, infant exposure, and occupational exposure. Sample sizes varied across and within endpoints (see Matric 4) but were sufficient to allow for statistical analysis. For several endpoints			
				(see Neure 4) but were sufficient to above for statistical analysis. For several endpoints, the authors noted that inter-assay coefficients of variability were $<10\%$.			

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Diethylhexyl Phthalate

		continued from previous page			
Study Citation:	Santacruz-Márquez, R., Safar, A. M., Laws, M effects of short-term and long-term phthalate ex 110(1):198-210.	M. J., Meling, D. D., Liu, Z., Kuma posures on ovarian follicle growth dy	r, T. R., Nowak, R. A., Raetzman, L. T., Flaws, J. A. (2024). The namics and hormone levels in female mice [†] . Biology of Reproduction		
Health Outcome(s)	Reproductive/Developmental-Ovary histopatho	logy, serum hormones (progesterone	e, testosterone, estradiol, FSH, LH), and gene expression in ovarian		
and Reported	tissue-Other (please specify below) (Endocrine)	-Gene expression in pituitary tissue			
Health Effect(s):					
Duration and	Oral-Diet-Duration: Chronic (>90 days)-7-6-m	ionth(s)			
Exposure Route:					
Species:	Mouse-CD-1 - [mouse]-Female				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	11784618				
Domain	Metric	Rating	Comments		
Additional Comments:	None				

Overall Quality Determination

Medium

Study Citation:	Akingbemi, steroidogeni	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.					
Health Outcome(s) and Reported	Reproductiv vesicles wei	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight					
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-1-F	F0 - gestation (C	GD 12-21)-F0- lactation			
Species:	Rat-Long-E	vans - [rat]-Female					
Chemical:	Diethylhexy	Diethylhexyl Phthalate- Parent compound					
HERO ID:	673553						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to "ensure equal weight distri- bution between groups". Male offspring were "randomly obtained from seven dams in each group at each stage," but the method of randomization was not specified.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food in- take, body weights, organ weights, hormone concentrations) or did not require blinding (histology)			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.			
Domain 4: Selective Re	porting and At	trition					
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.			
		Contin	ued on next pa	ge			

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Diethylhexyl Phthalate

		cont	inued from previ	ous page		
Study Citation: Health Outcome(s) and Reported	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight					
Health Effect(s):	Oral Causes Duration: Regarduative/Davalenmental 1 E0 . contation (CD 12 21) E0 . Instation					
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation					
Exposure Route: Species:	Rat-Long-Evans - [rat]-Female					
Chemical:	Diethylhexy	Rat-Long-Evans - [rat]-Female Diethylbexyl Phthalate- Parent compound				
HERO ID:	673553					
Domain		Metric	Rating	Comments		
Domain 5: Exposure M	lethods Sensitiv	vity				
Domain 5. Exposure M	Metric 6:	Chemical administration and	Low	The source and purity of the test substance was reported. Certificates of analysis are		
	incure of	characterization	2011	available from the supplier upon request; the test substance was reported. Certified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.		
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during GD 12-21 which is the relevant period for male sexual differen- tiation (Ema et al. 1993). Exposure was consistent across study groups. Groups were treated concurrently.		
Domain 6: Outcome M	essures and Re	eulte Dienlay				
Domain 6: Outcome M	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 414 Guideline which focuses on developmental toxicity recommends using more than 16 animals. In this study, there was a total of 7 dams and 9-18 offspring. The study authors did not justify the number of animals per group and sample sizes; however, sample sizes were sufficient to allow for statistical analysis. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Outcome methodologies for the offspring were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.		
	Metric 9:	Results presentation	Low	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs, however other endpoints (e.g., organ weights) were only reported as negative in the text and data were not shown. Statistical analysis methods were reported and statistical significance was noted in tables. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided. As noted by Dishaw et al., 2020, the presentation of offspring data as means of individual animals, rather than as litter means, has the potential to overestimate the statistical significance of experimental findings.		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation:	Akingbemi,	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell						
Health Outcome(s) and Reported	Reproductiv vesicles weig	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight						
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F0-	- lactation (PNI	D 1-21)				
Species: Chemical: HERO ID:	Rat-Long-Ev Diethylhexy 673553	vans - [rat]-Female l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.				
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to "ensure equal weight distri- bution between groups". Male offspring were "randomly obtained from seven dams in each group at each stage." but the method of randomization was not specified.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food in- take, body weights, organ weights, hormone concentrations) or did not require blinding (histology)				
		<i>.</i>						
Domain 5: Contounding	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.				
Domain 4: Selective Re	porting and At	trition						
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.				
Domain 5: Exposure M	ethods Sensitiv	ity						
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Diethylhexyl Phthalate

HERO ID: 673553 Table: 2 of 4

		conti	nued from previ	ous page			
Study Citation: Health Outcome(s) and Reported	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259. Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal variable.						
Health Effect(s):	vesieres wei	vesicles weight					
Duration and	Oral-Gavag	e-Duration: Reproductive/Developmental-F	0- lactation (PNI	D 1-21)			
Exposure Route:							
Species:	Rat-Long-E	vans - [rat]-Female					
HERO ID:	673553	I Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the lactation period, which was appropriate for the outcomes of in- terest. Exposure was consistent across study groups. Groups were treated concurrently.			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 414 Guideline which focuses on developmental toxicity recommends using more than 16 animals. In this study, there was a total of 7 dams and 9-18 offspring. The study authors did not justify the number of animals per group and sample sizes; however, sample sizes were sufficient to allow for statistical analysis. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Outcome methodologies for the offspring were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.			
	Metric 9:	Results presentation	Low	Results for developmental endpoints were shown in tables (shown as means \pm SEM) or graphs, however other endpoints (e.g., organ weights) were only reported as negative in the text and data were not shown. Results for body weight were presented in a table (shown as means \pm SEM). Food consumption results was not reported for this experiment. Statistical analysis methods were reported and statistical significance was noted in tables. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided. As noted by Dishaw et al., 2020, the presentation of off-spring data as means of individual animals, rather than as litter means, has the potential to overestimate the statistical significance of experimental findings.			
Additional Comments:	None						
Overall Quali	ty Deteri	mination	Medium				

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.							
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Poutco	Nutritional/I Oral-Gavage	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation						
Exposure Route: Species: Chemical: HERO ID:	Rat-Long-Evans - [rat]-Female Diethylhexyl Phthalate- Parent compound 673553							
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.				
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to "ensure equal weight distri- bution between groups". Male offspring were "randomly obtained from seven dams in each group at each stage" but the method of randomization was not specified				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food in- take, body weights, organ weights, hormone concentrations) or did not require blinding (histology)				
Domain 2: Confoundin	a / Variabla Ca	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates; This is not expected to have a significant impact on the endpoint of interest (e.g., body weights). Cages, food containers, and water dispensing containers were not described.				
Domain 4: Selective Re	eporting and At Metric 5:	trition Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.				
Domain 5: Exposure M	ethods Sensitiv	ity						
Continued on next page								

PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

		cont	inued from previ	ous page			
Study Citation:	Akingbemi, steroidogeni	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.					
Health Outcome(s) and Reported	Nutritional/N	Metabolic-Body weight and food consump	tion in dams and y	roung adult rats			
Health Effect(s): Duration and	Oral-Gavage	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation					
Exposure Route:	orar ourage		geotation (e				
Species:	Rat-Long-Ev	vans - [rat]-Female					
Chemical: HERO ID:	673553	I Phinalate- Parent compound					
Domain	0,0000	Metric	Rating	Comments			
2	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during GD 12-21 which is the relevant period for male sexual differen- tiation (Ema et al. 1993). Exposure was consistent across study groups. Groups were treated concurrently.			
Domain 6: Outcome Me	asures and Res	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The number of animals per group and sample size were appropriate for the outcome of interest. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted. The methods did not specify the timing of body weight and feed intake measurements, but the results noted body weights were measured at the beginning and end of the exposure.			
	Metric 9:	Results presentation	Medium	Dam body weight measurements were quantitatively reported as means and unspecified measures of variance. Food intake results were not reported.			
Additional Comments:	None						

Overall Quality Determination

Medium

Study Citation:	Akingbemi, steroidogeni	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylbexyl)phthalate. Biology of Reproduction 65(4):1252-1259						
Health Outcome(s) and Reported Health Effect(s):	Nutritional/N	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats						
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F0	- lactation (PNI	0 1-21)				
Species: Chemical: HERO ID:	Rat-Long-Ev Diethylhexy 673553	vans - [rat]-Female l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	Quality Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.				
Domain 2: Selection an	d Performance							
	Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to "ensure equal weight distri- bution between groups". Male offspring were "randomly obtained from seven dams in each group at each stage," but the method of randomization was not specified.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food in- take, body weights, organ weights, hormone concentrations) or did not require blinding (histology)				
Domain 3: Confounding	σ / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. This is not expected to have a sig- nificant impact on the endpoint of interest (body weight).				
Domain 4: Selective Re	eporting and At	trition						
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.				
Domain 5: Exposure M	lethods Sensitiv	ity						
Continued on next page								

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673553 Table: 4 of 4

		conti	inued from previ	ous page			
Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.						
Health Outcome(s) and Reported	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats						
Health Effect(s):							
Duration and	Oral-Gavage	e-Duration: Reproductive/Developmental-F	FO- lactation (PNI	0 1-21)			
Exposure Route:							
Species:	Rat-Long-E	vans - [rat]-Female					
Chemical:	Diethylhexy	Phthalate- Parent compound					
HERO ID:	673553						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during GD 12-21 which is the relevant period for male sexual differen- tiation (Ema et al. 1993). Exposure was consistent across study groups. Groups were treated concurrently.			
Domain 6: Outcome Me	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The number of animals per group and sample size were suitable for the outcome of interest. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted. The methods did not specify the timing of body weight and feed intake measurements, but the results noted body weights were measured at the beginning and end of the exposure.			
	Metric 9:	Results presentation	Medium	Dam body weight measurements were quantitatively reported as means and unspecified measures of variance. Food intake results were not reported.			
Additional Comments:	None						

Overall Quality Determination

Medium

Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-					
Health Outcome(s) and Reported Health Effect(s): Duration and	97. Reproductive/Developmental-Note: The study conducted separate experiments, not all relevant reproductive/developmental endpoints were assessed in each experiment. Endpoints include: sperm parameters (production and morphology, sertoli cell number and leptotene spermatocyte to sertoli cell ratio), serum testosterone, male reproductive organ weights (testis, epididymis, seminal vescicles and prostate), criptochidism, histopathology and morphometry (testis) fertility and time to mating, and mating and pregnancy indices, sexual behavior, gross morphology of male reproductive organs. Fetal endpoints: Fetal weights, live/dead fetuses, implantation sites, resorptions, sex, external examinations. Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)					
Exposure Route:	Dat Wiston	[mat] Famala				
Species: Chemical	Diethylbeyyl	Phthalate- Parent compound				
HERO ID:	673565	Thinanate-Tarent compound				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality	Deverting Orgality	Madiana			
	Metric 1.	Reporting Quanty	Medium	An erhical and some important miorination were provided. Fenale Wista rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities \geq 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 \pm 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (tem- perature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.		
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	It was not specified how dams were allocated into dose groups, and there is no indica- tion that methods were taken to minimize selection bias (e.g., normalization for body weights). However, a referenced study by the same group HERO 674171 did specify that females were randomly assigned, but the method was not reported. It is not clear though, that the same was done for this study. Randomization was included for specific endpoints. For testicular morphometry, ten randomly chosen fields were analyzed for each animal, and 20 round seminiferous tubule cross-sections were randomly chosen for each animal to count Sertoli cell nucleoli. The study included a male offspring mating experiment. It was not indicated whether the male offspring were randomly selected for mating on PND 110, or for sexual behavioral assessments.		
Continued on next page						

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 1 of 5

		continu	ued from previ	ous page			
Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-					
Health Outcome(s)	97. Reproductiv	ve/Developmental-Note: The study conducted	d separate expe	riments not all relevant reproductive/developmental endpoints were assessed in			
and Reported	each experiment. Endpoints include: sperm parameters (production and morphology, sertoli cell number and leptotene spermatocyte to sertoli cell ratio)						
Health Effect(s):	serum testo	sterone, male reproductive organ weights (tes	tis, epididymis,	seminal vescicles and prostate), criptochidism, histopathology and morphometry			
	(testis) ferti Fetal weigh	lity and time to mating, and mating and preg	nancy indices, s	sexual behavior, gross morphology of male reproductive organs. Fetal endpoints: rnal examinations			
Duration and	and Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)						
Species:	Rat-Wistar	- [rat]-Female					
Chemical:	Diethylhexy	vl Phthalate- Parent compound					
HERO ID:	673565						
Domain		Metric	Rating	Comments			
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded; the measurements for most of the endpoints in this outcome were not subjective in nature (e.g., measurements of serum testosterone, organ weights, or fell under initial histopathology examinations (morphometry examinations); however, at least one of the endpoints in this outcome could be subjective in nature (sertoli cell counts). The methods used for the cell counts (e.g., hemocytometer) were not specified.			
Domain 3: Confoundin	ng / Variable Co Metric 4:	ontrol Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments,			
				the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confound- ing factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.			
Domain 4: Selective R	eporting and A	ttrition					
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortal- ity, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers re- flected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes spec- ified in the methods were reported in a quantitative or qualitative manner.			

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 1 of 5

	continued from previous page
Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di. (2. ethylbeyd) phthalate (DEHP): Reproductive effects on adult male offspring rate. Toxicology 228(1):85-
	07
Health Outcome(s)	Reproductive/Developmental-Note: The study conducted separate experiments, not all relevant reproductive/developmental endpoints were assessed in
and Reported	each experiment. Endpoints include: sperm parameters (production and morphology, sertoli cell number and leptotene spermatocyte to sertoli cell ratio),
Health Effect(s):	serum testosterone, male reproductive organ weights (testis, epididymis, seminal vescicles and prostate), criptochidism, histopathology and morphometry
	(testis) fertility and time to mating, and mating and pregnancy indices, sexual behavior, gross morphology of male reproductive organs. Fetal endpoints:
	Fetal weights, live/dead fetuses, implantation sites, resorptions, sex, external examinations.
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)

	e .	· •	· · · · ·		
n and	Oral-Gavage-Duration:	Reproductive/Develo	pmental-F0 - gestation (From GD 6)-F0- lactation	(through LD 21)

Exposure Route:	
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673565

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.

Domain 6: Outcome Measures and Results Display

PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

... continued from previous page Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study **Study Citation:** following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-97. Health Outcome(s) Reproductive/Developmental-Note: The study conducted separate experiments, not all relevant reproductive/developmental endpoints were assessed in each experiment. Endpoints include: sperm parameters (production and morphology, sertoli cell number and leptotene spermatocyte to sertoli cell ratio), and Reported Health Effect(s): serum testosterone, male reproductive organ weights (testis, epididymis, seminal vescicles and prostate), criptochidism, histopathology and morphometry (testis) fertility and time to mating, and mating and pregnancy indices, sexual behavior, gross morphology of male reproductive organs. Fetal endpoints: Fetal weights, live/dead fetuses, implantation sites, resorptions, sex, external examinations. Duration and Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21) **Exposure Route:** Species: Rat-Wistar - [rat]-Female **Chemical:** Diethylhexyl Phthalate- Parent compound **HERO ID:** 673565 Domain Metric Rating Comments

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 1 of 5

		continued from previ	ious page		
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-97. Reproductive/Developmental-Note: The study conducted separate experiments, not all relevant reproductive/developmental endpoints were assessed in each experiment. Endpoints include: sperm parameters (production and morphology, sertoli cell number and leptotene spermatocyte to sertoli cell ratio), serum testosterone, male reproductive organ weights (testis, epididymis, seminal vescicles and prostate), criptochidism, histopathology and morphometry (testis) fertility and time to mating, and mating and pregnancy indices, sexual behavior, gross morphology of male reproductive organs. Fetal endpoints:				
Duration and Exposure Route: Species: Chemical: HERO ID:	 Fetal weights, live/dead fetuses, implantation sites, resorptions, sex, external examinations. Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21) Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 673565 				
Domain	Metric	Rating	Comments		
	Metric 9: Results presentation	Medium	The adequacy of the data reporting of reproductive effects varies by endpoint. Reproductive organ weights, and serum testosterone levels were reported quantitatively as means \pm SE. Statistical methods were adequately described. Likewise, daily sperm production and sperm and testicular morphometry, and testicular cell count data were quantitatively reported in an acceptable manner. In all cases, outliers (macroscopically small testes/epididymides, and one extremely enlarged testis) were excluded from the analysis. Reproductive performance data, fetal data (e.g., viable fetuses, fetal body weights), and sexual behavior data were all quantitatively reported with adequate descriptions of statistical analysis. Results for some reproductive endpoints were described qualitatively (reproductive tract malformation) or semi-quantitatively (testis-histopathology) in the text with a representative figure showing the observed effects for the latter. Incidences were not reported for every observed effect, although dose groups were specified. It does not appear that data for these endpoints were statistically analyzed and are not reported in a manner that would allow for an independent analysis.		
Additional Comments:	None				
Overall Quali	ity Determination	Medium	L		

Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative statement of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F0	- gestation (Fro	m GD 6)-F0- lactation (through LD 21)		
Species:	Rat-Wistar -	[rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	673565					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq 98\%$. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 \pm 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.		
Domain 2: Selection an	d Performance					
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indi- cation that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was miti- gated for these outcomes (organ weights) because the measurements were not subjective in nature.		
Domain 3: Confounding	g / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confound- ing factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.		
Domain 4: Selective Re	porting and At	trition				

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 2 of 5

		con	tinued from previ	ous page		
Study Citation:	Andrade, A. following in	J., Grande, S. W., Talsness, C. E., Gerich utero and lactational exposure to di-(2-eth	ke, C., Grote, K., (ylhexyl) phthalate	Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-		
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-	F0 - gestation (Fro	om GD 6)-F0- lactation (through LD 21)		
Species:	Rat-Wistar -	[rat]-Female				
Chemical: HERO ID:	Diethylhexy 673565	l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortal- ity, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers re- flected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes spec- ified in the methods were reported in a quantitative or qualitative manner.		
Domain 5: Exposure M	ethods Sensitiv	vity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.		

Domain 6: Outcome Measures and Results Display

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 2 of 5

		conti	inued from previ	ious page		
Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F	F0 - gestation (Fro	om GD 6)-F0- lactation (through LD 21)		
Species: Chemical: HERO ID:	Rat-Wistar - Diethylhexy 673565	- [rat]-Female I Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 8:	Endpoint sensitivity and specificity	Low	Organ weights (liver, kidney, spleen, and thymus) alone, in the absence of histopathol- ogy, is not the most sensitive endpoint for identifying organ-specific toxicity; however, the purpose of the study was to evaluate reproductive effects and recording the weights of these other organs seemed to be an aside. The methods are generally adequately de- scribed; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 \pm 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't spec- ified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a sig- nificant impact on the organ weight results and makes it difficult to interpret the results reported. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple out- comes.		
	Metric 9:	Results presentation	Medium	Negative effects on liver, kidney, spleen, and thymus organ weights were qualitatively reported in the text. Based on the methods, statistical analysis for liver and kidney weights was analyzed with body weight as a co-variate, but the spleen and thymus were evaluated without adjustment due to an absence of meaningful correlation between those organs and body weight. Magnitudes of changes were not reported, so it cannot be determined whether there were any biologically relevant changes.		
Additional Comments:	None					

Overall Quality Determination

Diethylhexyl Phthalate

Medium

Study Citation:	Andrade, A. following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative st ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F0	- gestation (Fro	m GD 6)-F0- lactation (through LD 21)		
Species:	Rat-Wistar -	[rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	673565					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality					
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq 98\%$. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 \pm 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.		
Domain 2: Selection an	d Performance					
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indi- cation that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was miti- gated for these outcomes (organ weights) because the measurements were not subjective in nature.		
Domain 3: Confounding	n / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confound- ing factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.		
Domain 4: Selective Re	porting and At	trition				

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 3 of 5

		cont	inued from previ	ous page		
Study Citation:	Andrade, A. following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Live ment of nega	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)				
Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)				
Species: Chemical: HERO ID:	Rat-Wistar - Diethylhexy 673565	[rat]-Female l Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortal- ity, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers re- flected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes spec- ified in the methods were reported in a quantitative or qualitative manner.		
Domain 5: Exposure M	ethods Sensitiv	rity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq 98\%$, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.		

Domain 6: Outcome Measures and Results Display

Continued on next page ...

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 3 of 5

		conti	inued from previ	ious page	
Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-			
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)				
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F	F0 - gestation (Fro	om GD 6)-F0- lactation (through LD 21)	
Species: Chemical: HERO ID:	Rat-Wistar - Diethylhexy 673565	- [rat]-Female I Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 8:	Endpoint sensitivity and specificity	Low	Organ weights (liver, kidney, spleen, and thymus) alone, in the absence of histopathol- ogy, is not the most sensitive endpoint for identifying organ-specific toxicity; however, the purpose of the study was to evaluate reproductive effects and recording the weights of these other organs seemed to be an aside. The methods are generally adequately de- scribed; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 \pm 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't spec- ified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a sig- nificant impact on the organ weight results and makes it difficult to interpret the results reported. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple out- comes.	
	Metric 9:	Results presentation	Medium	Negative effects on liver, kidney, spleen, and thymus organ weights were qualitatively reported in the text. Based on the methods, statistical analysis for liver and kidney weights was analyzed with body weight as a co-variate, but the spleen and thymus were evaluated without adjustment due to an absence of meaningful correlation between those organs and body weight. Magnitudes of changes were not reported, so it cannot be determined whether there were any biologically relevant changes.	
Additional Comments:	None				

Overall Quality Determination

Diethylhexyl Phthalate

Medium

Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-			
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)				
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F0	- gestation (Fro	om GD 6)-F0- lactation (through LD 21)	
Species:	Rat-Wistar -	- [rat]-Female			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	6/3565				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities \geq 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 ± 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indi- cation that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was miti- gated for these outcomes (organ weights) because the measurements were not subjective in nature.	
Domain 3: Confounding	y / Variable Co	ontrol			
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confound- ing factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.	
Domain 4: Selective Re	porting and At	ttrition			
Continued on next page					

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 4 of 5

		con	tinued from previ	ous page		
Study Citation:	Andrade, A. following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Live ment of nega	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)				
Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)				
Species:	Rat-Wistar -	Rat-Wistar - [rat]-Female				
Chemical: HERO ID:	673565	I Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortal- ity, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers re- flected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes spec- ified in the methods were reported in a quantitative or qualitative manner.		
Domain 5: Exposure M	ethods Sensitiv	vity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.		

Domain 6: Outcome Measures and Results Display

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673565 Table: 4 of 5

		cont	inued from previ	ous page	
Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-			
Health Outcome(s) and Reported Health Effect(s):	97. Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative state- ment of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)				
Exposure Route:	Ofai-Oavag	e-Duration. Reproductive/Developmental-r	o - gestation (140		
Species:	Rat-Wistar -	- [rat]-Female			
Chemical: HERO ID:	673565	Phthalate- Parent compound			
Domain		Metric	Rating	Comments	
	Metric 8:	Endpoint sensitivity and specificity	Low	Organ weights (liver, kidney, spleen, and thymus) alone, in the absence of histopathol- ogy, is not the most sensitive endpoint for identifying organ-specific toxicity; however, the purpose of the study was to evaluate reproductive effects and recording the weights of these other organs seemed to be an aside. The methods are generally adequately de- scribed; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 \pm 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't spec- ified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a sig- nificant impact on the organ weight results and makes it difficult to interpret the results reported. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple out- comes.	
	Metric 9:	Results presentation	Medium	Negative effects on liver, kidney, spleen, and thymus organ weights were qualitatively reported in the text. Based on the methods, statistical analysis for liver and kidney weights was analyzed with body weight as a co-variate, but the spleen and thymus were evaluated without adjustment due to an absence of meaningful correlation between those organs and body weight. Magnitudes of changes were not reported, so it cannot be determined whether there were any biologically relevant changes.	
Additional Comments:	None				

Overall Quality Determination

Diethylhexyl Phthalate

Medium

Study Citation:	Andrade, A.	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported	97. Nutritional/Metabolic-Body weights (off juvenile/adult male offspring)					
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)				
Species:	Rat-Wistar -	Rat-Wistar - [rat]-Female				
Chemical:	Diethylhexy	Diethylhexyl Phthalate- Parent compound				
HERO ID:	673565					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Qu	uality					
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities \geq 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 ± 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.		
Domain 2: Selection and	l Performance					
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indi- cation that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was miti- gated for this outcome (body weights) because the measurements were not subjective in nature.		
Domain 3: Confounding	Domain 3: Confounding / Variable Control					
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confound- ing factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.		
Domain 4: Selective Rep	porting and At	trition				
		Contin	ued on next pa	nge		

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Diethylhexyl Phthalate

		co	ntinued from previo	us page		
Study Citation:	Andrade, A. following in u	J., Grande, S. W., Talsness, C. E., Geri atero and lactational exposure to di-(2-et	cke, C., Grote, K., G thylhexyl) phthalate (olombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-		
Health Outcome(s) and Reported Health Effect(c):	97. Nutritional/M	97. Nutritional/Metabolic-Body weights (off juvenile/adult male offspring)				
Duration and Exposure Route:	Oral-Gavage-	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)				
Species:	Rat-Wistar -	[rat]-Female				
Chemical: HERO ID:	673565	Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortal- ity, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers re- flected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes spec- ified in the methods were reported in a quantitative or qualitative manner.		
Domain 5: Exposure Me	thods Sensitivi	ty				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.		
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.		

Domain 6: Outcome Measures and Results Display

May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

		conti	nued from previ	ious page		
Study Citation:	Andrade, A following in	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-				
Health Outcome(s) and Reported Health Effect(s):	Nutritional/	Nutritional/Metabolic-Body weights (off juvenile/adult male offspring)				
Duration and Exposure Route:	Oral-Gavag	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)				
Species:	Rat-Wistar	- [rat]-Female				
Chemical:	Diethylhexy	Diethylhexyl Phthalate- Parent compound				
Domain	073505	Metric	Rating	Comments		
	Metric 8: Metric 9:	Endpoint sensitivity and specificity Results presentation	Low	The endpoint (body weights of male offspring) was sensitive, but it is unclear why body weights were not measured throughout the study instead of singular measurements of final body weights. There were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 \pm 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't specified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a significant impact on the body weight results and makes it difficult to interpret the results reported. The number of animals used was appropriate for statistical analysis. Body weight data were quantitatively reported as means \pm SE. Statistical methods were		
				described and were adequate.		
Additional Comments:	None					
Overall Qualit	ty Deteri	nination	Medium	l		

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Andrade, A. utero and lac male offsprin Reproductive anogenital d weights on F Oral-Gavage Rat-Wistar - Diethylhexy 673567	Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225(1):64-74. Reproductive/Developmental-Developmental effects: number of live and dead pups, pup body weights, sex, general signs of toxicity, nipple retention, anogenital distance, age of testes decent, external examinations of reproductive organs for malformations, histopathology of the testes, liver and brain weights on PND1, liver, brain, testis and epididymis weights on PND 22. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (From GD 6)-F0- lactation (through PND 21) Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 673567			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Qu	uality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Dams (11-16 per group) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through PND 21. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities \geq 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 ± 15g). The parity and age of the animals were not specified. HERO ID 674171 (which published the female data for this study) was referenced for animal husbandry conditions, were all reported along with the number of animals per cage, Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Medium	The referenced study HERO 674171 indicated dams were randomly assigned into groups, but the method of randomization was not specified. It is unknown whether animals were normalized for body weight. The current study also indicated that 1 or 2 male pups were randomly selected from each litter for sacrifice on PND1. The method of randomization also was not specified.	
	Metric 3:	Observational Bias / Blinding Changes	Low	The study indicated that anogenital distance was measured in a blinded manner and that initial histopathology was also done blinded and there was no secondary histopatholog- ical evaluation. Blinding was not required for other endpoints because they were not subjective in nature.	
Domain 3: Confounding	r / Variable Cou	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. All of the control re- sponses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were monitored and reported in HERO ID 674171 and there were no significant changes that were suggestive of palatability issues. Animal husbandry details reported in the same referenced study were consistent across groups.	
Domain 4: Selective Rep	porting and Att	trition			

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673567 Table: 1 of 1

		cont	inued from previ	ous page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225(1):64-74. Reproductive/Developmental-Developmental effects: number of live and dead pups, pup body weights, sex, general signs of toxicity, nipple retention, anogenital distance, age of testes decent, external examinations of reproductive organs for malformations, histopathology of the testes, liver and brain weights on PND1, liver, brain, testis and epididymis weights on PND 22. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (From GD 6)-F0- lactation (through PND 21) Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 673567			
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	HERO ID 674171 was referenced for information on dams. The number of litters per group varied (11-16), and this reference indicates this is also the number of dams. It is not clear why there are such differences between groups (e.g., if a common number was intended, but some dams died, or turned out not to be gravid). The study also did not report the total number of pups born, and viability between birth and PND22 was not reported. The number of pups sampled for different endpoints varied making it difficult to know whether there was evidence of attrition, or if the data were selectively reported. On PND 22 at the high dose, liver, brain, and epididymis weights were only obtained from 13 animals (7 litters), even though body weights were measured in 15 pups from 8 litters. The study authors did not describe the reasoning for the difference. Qualitative or quantitative data were reported for all of the specified outcomes and endpoints.
Domain 5: Exposure M	lethods Sensitiv	vity		
Domani J. Exposure M	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities \geq 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurement were conducted (e.g., only once, or with each preparation etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population, and the highest dose was known to induce adverse reproductive effects in male offspring without causing over maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.

Domain 6: Outcome Measures and Results Display

Continued on next page ...

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 673567 Table: 1 of 1

		conti	nued from previ	ous page
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Andrade, A utero and la male offspri Reproductiv anogenital o weights on Oral-Gavag Rat-Wistar Diethylhexy 673567	J., Grande, S. W., Talsness, C. E., Grote, inctational exposure to di-(2-ethylhexyl) pht ing rats. Toxicology 225(1):64-74. /e/Developmental-Developmental effects: 1 distance, age of testes decent, external exa PND1, liver, brain, testis and epididymis we e-Duration: Reproductive/Developmental-1 - [rat]-Female /l Phthalate- Parent compound	K., Golombiewsk halate (DEHP): E number of live ar minations of repr eights on PND 22 -F0 - gestation (F	i, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following in ffects on androgenic status, developmental landmarks and testicular histology in d dead pups, pup body weights, sex, general signs of toxicity, nipple retention, oductive organs for malformations, histopathology of the testes, liver and brain rom GD 6)-F0- lactation (through PND 21)
Domain		Metric	Rating	Comments
	Metric 8: Metric 9:	Endpoint sensitivity and specificity Results presentation	Medium	The endpoints were sensitive and specific for the outcomes of interest and were con- sistent with the purpose of the study which was to evaluate the sexual development of male offspring from birth to puberty, with a focus on androgen-sensitive endpoints. Ani- mals in each group were consistently sacrificed on the same day. The sample sizes were appropriate and sufficient for performing statistical analysis and NOAEL and LOAEL values were determined, although histopathology was only performed on 4-6 pups per dose, which may have reduced the statistical power for that endpoint since no signif- icant changes were observed. The authors provided justification for the doses chosen (based on the median daily intake of the general German population and doses previ- ously shown to induce adverse effects in male offspring). Data for all endpoints were presented quantitatively as means \pm SE, or as incidences (histopathology), which included measures of severity. The methods of statistical analy-
				sis were described and were adequate. It does not appear that histopathology data were statistically analyzed, but sufficient data are provided for an independent analysis.
Additional Comments:	publication	for this study. These data are quantitatively	n maternal weigh (weight gain) rep	orted in HERO ID 674171; therefore, maternal data were not evaluated here.
Overall Quali	ty Deteri	mination	Medium	

PUBLIC RELEASE DRAFT

Study Citation: Health Outcome(s)	Culty, M., Thuillier, R., Li, W., Wang, Y., Martinez-Arguelles, D., Benjamin, C., Triantafilou, K., Zirkin, B., Papadopoulos, V. (2008). In utero exposure to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat. Biology of Reproduction 78(6):1018-1028. Reproductive/Developmental-Testosterone production in ex vivo fetal organ cultures.						
and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	Oral-Gavage Rat-Sprague- Diethylhexyl	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14 - parturition) Rat-Sprague-Dawley - [rat]-Female Diethylbeyyl Phthalate- Parent compound					
HERO ID:	698207						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Qu	aality Metric 1:	Reporting Quality	Low	All critical and some important information was reported. Reported information in- cluded information on the test substance (name and source), the test model (species, strain, sex, and source), animal husbandry details (photoperiod, food and water avail- ability), exposure methods, experimental design, endpoint evaluations, and presentation of results. Missing information included the purity of the test substance, test animal age, initial body weights, parity, additional animal husbandry details (temperature, humidity, number of animals per cage), and the number of dams per treatment group.			
Domain 2: Selection and	l Performance						
	Metric 2:	Allocation	Low	No details on the allocation of dams into study groups or on the selection of fetuses for outcome analysis were provided.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcome was measured using a standard laboratory kit.			
Domain 3: Confounding	/ Variable Con	trol					
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. Consistency of other potentially con- founding factors (e.g., most animal husbandry conditions, body weights, food or water intake, or gavage volume) were not reported. It is unclear whether the study took mea- sures to minimize the exposure to other plasticizers which could influence the study results. No information on cage type or other materials used in the study were provided.			
Domain 4: Selective Ret	porting and Att	rition					
	Metric 5:	Selective Reporting and Attrition	Medium	The total number of dams included in each test group was not specified. The data relevant to the endpoint of interest was derived from $n = 3$ litters. The study did not report endpoints that would allow for the determination of possible attrition.			
Domain 5: Exposure Me	ethods Sensitivi	ty					
Continued on next page							

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 698207 Table: 1 of 1

		cont	tinued from p	previous page			
Study Citation:	Culty, M., Thuillier, R., Li, W., Wang, Y., Martinez-Arguelles, D., Benjamin, C., Triantafilou, K., Zirkin, B., Papadopoulos, V. (2008). In utero expo to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat. Biology of Reproduce 78(6):1018-1028.						
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testosterone production in ex vivo fetal organ cultures.						
Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14 - parturition) te:						
Species: Chemical: HERO ID:	Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 698207						
Domain		Metric	Rating	Comments			
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma) was reported. The purity was not specified, and no certificate of analysis was provided in the study report. Purity and certificates of analysis would have been available on the supplier's website and the time of purchase. There is no indication that the test substance was verified by the performing laboratory. Animals were dosed via gavage in corn oil and the gavage volume was not reported. No details on the preparation, storage, or stability of the test solutions were provided. The study included 4 dose groups plus a control. Doses were reported in mg/kg-day and were adjusted daily based dam body weights, although the frequency of measurements was not specified. Doses were not analytically verified.			
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD14 until parturition. This exposure covers the period of post-implantation embryonic development and the critical windows of organogenesis and male sexual differentiation.			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	Low	There are no major concerns regarding the specificity and validity of the protocol for measuring testosterone production; however, some details were missing. Testes (1 or 2 not specified) from GD20 fetuses (number not specified) from 3 litters per dose were cultured ex vivo for 1 or 2 days, or for 3 days with or without the addition of hCG. Testosterone production was measured using an ELISA assay. The dose spacing did not allow for the determination of a NOAEL for this endpoint. The test species and strain were appropriate for the study type.			
	Metric 9:	Results presentation	High	Results were reported in a figure (bar graph) showing means \pm SEM. Statistical significance and sample size (n=3 litters) were shown. The litter was used as the experimental unit. Individual animal data were not provided.			
Additional Comments:	Only fetal to	estosterone was evaluated for data quality.	Testosterone	levels in neonates or adult offspring were not evaluated.			
	4 D-4	······································	T				
Overall Quall	iy Deterl	IIIIIauon	LOW				

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Study Citation: Health Outcome(s) and Reported Health Effect(c):	Gray, L. E., Hormonal E Unique Adv Reproductiv	Gray, L. E., Jr, Lambright, C. S., Conley, J. M., Evans, N., Furr, J. R., Hannas, B. R., Wilson, V. S., Sampson, H., Foster, D., P.M. (2021). Genomic and Hormonal Biomarkers of Phthalate-Induced Male Rat Reproductive Developmental Toxicity Part II: A Targeted RT-qPCR Array Approach That Defines a Unique Adverse Outcome Pathway. Toxicological Sciences 182(2):195-214. Reproductive/Developmental-Fetal testosterone production ex vivo						
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-GD18)							
Species:								
Chemical:	Diethylhexyl Phthalate- Parent compound							
HERO ID:	9419406 Linked HERO ID(s): 9419406, 12162058							
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	Quality							
	Metric 1:	Reporting Quality	High	All critical and important information is reported. The test chemical was identified by name and CASRN. The source, lot, catalogue number, and purity are provided in a supplemental file by Fur et al. (2014). Other reported information includes test animal details (species, strain, source, age, initial body weights, and parity), animal husbandry details (number per cage, food and water availability, photoperiod, temperature, and humidity), exposure methods, experimental design, endpoint evaluations, and presentation of results.				
Domain 2: Selection ar	nd Performance							
Domain 2. Selection a	Metric 2:	Allocation	Medium	The authors stated that pregnant dams were randomly assigned to treatment groups on GD14 in a manner that provided each group with similar means and variances in body weight. The method of randomization was not specified.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	The paper did not indicate that whether investigators were blinded during outcome as- sessment. However, the outcome of interest was measured using standard laboratory kits.				
Domain 3: Confoundin	og / Variable Co	ntrol						
	Metric 4:	Confounding / Variable Control	Medium	Vehicle (laboratory-grade corn oil) and gavage volume were the same in the control and treatment groups. Animals were housed individually. The study did not specify whether measures were taken to reduce the potential for exposure to plasticizers, which could influence study results in a study focused on assessing the potential for endocrine disruption. Municipal drinking water was tested monthly for Pseudomonas and every 4 months for a suite of chemicals including pesticides and heavy metals. However, the materials used to dispense water to animals were not specified and it was not reported whether food was tested for phthalate contamination. Animals were housed in polycarbonate rather than metal cages. The experimental conditions described provided no indication of different practices across treatment groups.				
Domain 4: Selective R	enorting and A	trition						
Zomani i. Selective K	Metric 5:	Selective Reporting and Attrition	High	Quantitative data for the endpoint of interest were provided and all of the litters were accounted for. There is no evidence suggesting attrition or selective reporting				

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 9419406 Table: 1 of 1

		cont	inued from p	revious page				
Study Citation:	Gray, L. E., Jr, Lambright, C. S., Conley, J. M., Evans, N., Furr, J. R., Hannas, B. R., Wilson, V. S., Sampson, H., Foster, D., P.M. (2021). Genomic and Hormonal Biomarkers of Phthalate-Induced Male Rat Reproductive Developmental Toxicity Part II: A Targeted RT-qPCR Array Approach That Defines a Unique Adverse Outcome Pathway. Toxicological Sciences 182(2):195-214.							
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Fetal testosterone production ex vivo							
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-GD18)							
Species:	Rat-Other (Crl:(CD)SD)-Female							
Chemical:	Diethylhexy	Diethylhexyl Phthalate- Parent compound						
HERO ID:	9419406 Linked HERO ID(s): 9419406, 12162058							
Domain		Metric	Rating	Comments				
Domain 5: Exposure M	Iethods Sensiti	vity						
	Metric 6:	Chemical administration and characterization	Medium	The test substance source, catalogue number, lot number, and purity (>99%) was reported (Furr et al. 2014). The test substance was not analytically verified by the performing laboratory. No details of preparation or storage of the test solutions were provided. The doses were clearly reported and were adjusted daily based on dam body weights. The gavage volume (2.5 mL/kg) was appropriate. Concentrations of the test substance in the dosing solutions was not analytically verified.				
	Metric 7:	Exposure timing, frequency, and duration	High	Pregnant dams were dosed daily from GD14-GD18. The authors reported this as a critical period of sexual differentiation. This paper was a continuation of a previous publication (Furr et al. 2014) and maintained the same exposure details.				
Domain 6: Outcome M	leasures and Re	esults Display						
	Metric 8:	Endpoint sensitivity and specificity	High	No concerns regarding the specificity and validity of the protocols and measures were identified. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. Samples were incubated individually for 3 hours. Measurements were collected from 1 testis/male from 3 males/litter from 3-4 litters.				
	Metric 9:	Results presentation	High	Results for testosterone production are shown in Figure 2. The figure does not specify the sample size and is reported as a % of control so lacks measures of variance. How- ever, raw data are available in the supplemental files. There are no notable concerns about the way the results are analyzed.				
Additional Comments:	Only fetal t	estosterone was evaluated for data quality.						
Overall Quali	ity Deter	mination	High					
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical:	 Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Reproductive/Developmental-Male Reproductive - testosterone Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18) Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 							
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Domain	788239	Metric	Rating	Comments				
Domain 1: Reporting O	uality	Weute	Kaung	Comments				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance identity and source were reported. Purity or grade were not reported. Test animal species, strain, sex, and source were reported. Age and initial body weights were not reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were housed individually. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.				
Domain 2: Selection and	d Performance							
Domain 2. Selection and	Metric 2:	Allocation	Medium	"Dams were weight ranked and assigned to dose groups to minimized differences in means and variance among treatment groups". It is not clear whether this was done randomly, but this description indicates that normalization procedures were performed to balance important variables across groups. The first three males identified from each litter were selected for measuring ex vivo testicular testosterone production.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, hormone levels, body weight) or clinical signs.				
Domain 3: Confounding	r / Variable Cor	atral						
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and appropriate. There were no indications that husbandry conditions were different between the groups. Food and water intake were not reported in an oral gavage study. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which has been linked to developmental and reproductive health problems. This could potentially confound results, although if control animals were exposed to the same levels, this may not substantially impact the interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plas- tic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophos- phates. No analysis of food for potential endocrine disruptors was conducted.				

Continued on next page ...

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 788239 Table: 1 of 6

		cont	inued from previ	ous page	
Study Citation:	Hannas, B. and gene ex phthalate. T	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216.			
Health Outcome(s) and Reported Health Effect(s):	Reproductiv	e/Developmental-Male Reproductive - test	osterone		
Duration and Exposure Route:	Oral-Gavag	e-Duration: Reproductive/Developmental-	1-F0 - gestation (C	GD 14-18)	
Species: Chemical: HERO ID:	Rat-Other (S Diethylhexy 788239	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 788239			
Domain		Metric	Rating	Comments	
Domain 4: Selective Re	eporting and At	trition			
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from $n = 3$ controls and $n = 4$ for all other dose groups. However, the sample sizes for T production were from $n = 6, 3, 3, 6, 4, 4$, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.	
Domain 5: Exposure M	ethods Sensitiv	zity			
	Metric 6:	Chemical administration and characterization	Medium	The purity or grade of the test substance was not reported but Sigma's website indicates a purity of $>98\%$. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure the concentrations in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.	
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. Preg- nant dams were dosed daily from GD 14-18, which coincides with the critical window	

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 788239 Table: 1 of 6

		cont	tinued from previo	us page		
Study Citation:	Hannas, B. and gene ex phthalate. T	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216				
Health Outcome(s) and Reported Health Effect(s): Duration and	Reproductiv Oral-Gavage	Oral Gavage Duration: Reproductive/Developmental 1 E0. gestation (GD 14 18)				
Exposure Route:						
Species: Chemical: HERO ID:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 788239					
Domain		Metric	Rating	Comments		
	Metric 8: Metric 9:	Endpoint sensitivity and specificity Results presentation	Medium High	The endpoints evaluated were sensitive to outcomes of interest. No concerns regarding the specificity of the protocols and measures were identified. qPCR samples were run in duplicate only, and it doesn't appear that there were any independent experimental replicates. It is not clear that cDNA levels were measured. An RNA to cDNA ratio of 1:1 was assumed. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. One testis each was dissected from the first 3 male fetuses/litter. The remaining testes were pooled to evaluate the expression of insl3, StAR, and Cyp11a. It is not clear whether the in- dividual testes used in the testosterone assay were left or right, so differential/bilateral effects are not evaluated. Sample size is small (n=3 dams/dose group), but was validated by the authors to have sufficient statistical power to evaluate changes in fetal testos- terone production, although authors stated that changes less than 20-25% may not be consistently detected (see Furr et al. 2014 [2510906]). Data were presented as means \pm SE. The "n" is assumed to be the number of litters assessed. Statistical analysis was performed by authors and was appropriate.		
	N					
Additional Comments:	INOne					
Overall Quali	ty Deteri	mination	Medium			

Duration and Oral-Gavage Exposure Route: Species: Species: Rat-Other (S Chemical: Diethylhexy HERO ID: 788239 Domain Domain 1: Reporting Quality Metric 1: Metric 1:	e-Duration: Reproductive/Developmental-1-F Sprague-Dawley- Charles River)-Female-Rat- l Phthalate- Parent compound Metric	F0 - gestation (C	GD 14-18) Female
Domain Domain 1: Reporting Quality Metric 1:	Metric		
Domain 1: Reporting Quality Metric 1:		Rating	Comments
	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance identity and source were reported. Purity or grade were not reported. Test animal species, strain, sex, and source were reported. Age and initial body weights were not reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were housed individually. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance Metric 2:	Allocation	Medium	"Dams were weight ranked and assigned to dose groups to minimized differences in means and variance among treatment groups". It is not clear whether this was done randomly, but this description indicates that normalization procedures were performed to balance important variables across groups. The first three males identified from each litter were selected for measuring ex vivo testicular testosterone production.
Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, hormone levels, body weight) or clinical signs.
Domain 3 [,] Confounding / Variable Co	ntrol		
Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and appropriate. There were no indications that husbandry conditions were different between the groups. Food and water intake were not reported in an oral gavage study. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which has been linked to developmental and reproductive health problems. This could potentially confound results, although if control animals were exposed to the same levels, this may not substantially impact the interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plas- tic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophos- phates. No analysis of food for potential endocrine disruptors was conducted.
Domain 4: Selective Reporting and At	trition		

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 788239 Table: 2 of 6

		conti	nued from previ	ious page		
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Reproductive/Developmental-Male Reproductive - testosterone					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-1	-F0 - gestation (C	GD 14-18)		
Species: Chemical: HERO ID:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound					
Domain	100237	Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from $n = 3$ controls and $n = 4$ for all other dose groups. However, the sample sizes for T production were from $n = 6, 3, 3, 6, 4, 4, and 3$ litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.		
Domain 5: Exposure N	Aethods Sensitiv	vity				
Domain C. Dapooure it	Metric 6:	Chemical administration and characterization	Medium	The purity or grade of the test substance was not reported but Sigma's website indicates a purity of $>98\%$. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure the concentrations in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.		
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. Preg- nant dams were dosed daily from GD 14-18, which coincides with the critical window of male sexual differentiation (Dent et al. 2015 [3452649]; Scott et al. 2009 [673313]).		
Domain 6: Outcome M	lancuras and Da	culte Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The endpoints evaluated were sensitive to outcomes of interest. No concerns regarding the specificity of the protocols and measures were identified. qPCR samples were run in duplicate only, and it doesn't appear that there were any independent experimental replicates. It is not clear that cDNA levels were measured. An RNA to cDNA ratio of 1:1 was assumed. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. One testis each was dissected from the first 3 male fetuses/litter. The remaining testes were pooled to evaluate the expression of insl3, StAR, and Cyp11a. It is not clear whether the individual testes used in the testosterone assay were left or right, so differential/bilateral effects are not evaluated. Sample size is small (n=3 dams/dose group), but was validated by the authors to have sufficient statistical power to evaluate changes in fetal testos-terone production, although authors stated that changes less than 20-25% may not be consistently detected (see Furr et al. 2014 [2510906]).		
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			. continued from previo	us page		
Study Citation:	Hannas, B. and gene ex phthalate. T	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216.				
Health Outcome(s)	Reproductiv	ve/Developmental-Male Reproductive	e - testosterone			
and Reported						
Health Effect(s):						
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)					
Exposure Route:						
Species:	Rat-Other (Sprague-Dawley- Charles River)-Fen	nale-Rat-Wistar - [rat]-Fe	omale		
Chemical:	Diethylhexy	yl Phthalate- Parent compound				
HERO ID:	788239					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	High	Data were presented as means \pm SE. The "n" is assumed to be the number of litters		
				assessed. Statistical analysis was performed by authors and was appropriate.		
Additional Comments:	None					
Overall Quali	ty Deteri	mination	Medium			

Study Citation:	Hannas, B.	R., Lambright, C. S., Furr, J., Howdeshell, K. L.	, Wilson, V. S., Gray	, L. E. (2011). Dose-response assessment of fetal testosterone production			
·	and gene ex phthalate. T	and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216.					
Health Outcome(s)	Mortality-M	Iortality (results reported for DINP and DIBP on	ly)-Other (please sp	ecify below) (Clinical signs)-Overt toxicity (results reported for DINP and			
and Reported	DIBP only)						
Health Effect(s):							
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)						
Exposure Route:							
Species:	Rat-Other (S	Sprague-Dawley- Charles River)-Female-Rat-Wi	istar - [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	788239						
Domain		Metric	Rating	Comments			
Domain 1: Reporting (Quality						
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance identity and source were reported. Purity or grade were not reported. Test animal species, strain, sex, and source were reported. Age and initial body weights were not reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were housed individually. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.			
Dennein 2. Selection e	- 1 D						
Domain 2: Selection a	nd Performance Metric 2:	Allocation	Medium	"Dams were weight ranked and assigned to dose groups to minimized differences in means and variance among treatment groups". It is not clear whether this was done randomly, but this description indicates that normalization procedures were performed to balance important variables across groups. The first three males identified from each litter were selected for measuring ex vivo testicular testosterone production.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, hormone levels body weight) or clinical signs.			
Domain 2: Confoundi	va / Variabla Ca	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and appropriate. There were no indications that husbandry conditions were different between the groups. Food and water intake were not reported in an oral gavage study. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which has been linked to developmental and reproductive health problems. This could potentially confound results; although if control animals were exposed to the same levels, this may not substantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plas- tic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophos- phates. No analysis of food for potential endocrine disruptors was conducted.			
D							
Domain 4: Selective R	eporting and At	trition					

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 788239 Table: 3 of 6

		c	ontinued from previous p	age		
Study Citation:	Hannas, B. I and gene ex	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216				
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality (results reported for DINP and DIBP only)-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only) DIBP only)					
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-1-	F0 - gestation (GD 14-18)			
Species: Chemical: HERO ID:	Rat-Other (S Diethylhexy 788239	Sprague-Dawley- Charles River)-Female-Ra l Phthalate- Parent compound	t-Wistar - [rat]-Female			
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from $n = 3$ controls and $n = 4$ for all other dose groups. However, the sample sizes for T production were from $n = 6, 3, 3, 6, 4, 4, and 3$ litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.		
Domain 5: Exposure M	ethods Sensitiv	áty.				
Domain 5: Exposure M	Metric 6:	Chemical administration and characterization	Medium	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.		
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure from GD 14-18 occurs at the end of the critical window of organogenesis and does not include pre-mating or early gestational stages, so may be less sensitive for evaluating maternal effects and effects on fetal survival and growth.		
Domain 6: Outcome M	ansuras and Da	sulte Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods did not specify that dams were observed for mortality or clinical signs and no results for these endpoints were reported for animals treated with DEHP; however, results for these endpoints were reported for animals treated with other chemicals (e.g., DINP, DIBP) and therefore, it is assumed that these endpoints were evaluated in all animals in the study. No details were provided on the frequency of observations.		
	Metric 9:	Results presentation	Uninformative	No results for mortality or clinical observations were provided in the text.		
Additional Comments:	None					
Overall Quali	ty Deterr	nination	Uninformative			

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HERO ID: 788239 Table: 3 of 6

		continued from previous page			
Study Citation:	Hannas, B. R., Lambright, C. S., Furr, J., Howdesl and gene expression levels in rat testes following phthalate. Toxicological Sciences 123(1):206-216.	nell, K. L., Wilson, V. S., Gray, L. E. (2011) in utero exposure to diethylhexyl phthalate). Dose-response assessment of fetal testosterone production e, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl		
Health Outcome(s)	Mortality-Mortality (results reported for DINP and	I DIBP only)-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and		
and Reported	DIBP only)				
Health Effect(s):					
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)				
Exposure Route:					
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	788239				
Domain	Metric	Rating	Comments		

Study Citation:	Hannas, B.	R., Lambright, C. S., Furr, J., Howdeshell, K. L.	, Wilson, V. S., Gray	, L. E. (2011). Dose-response assessment of fetal testosterone production			
·	and gene ex phthalate. T	and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216.					
Health Outcome(s)	Mortality-M	Iortality (results reported for DINP and DIBP on	ly)-Other (please sp	ecify below) (Clinical signs)-Overt toxicity (results reported for DINP and			
and Reported	DIBP only)						
Health Effect(s):							
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)						
Exposure Route:							
Species:	Rat-Other (S	Sprague-Dawley- Charles River)-Female-Rat-Wi	istar - [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	788239						
Domain		Metric	Rating	Comments			
Domain 1: Reporting (Quality						
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance identity and source were reported. Purity or grade were not reported. Test animal species, strain, sex, and source were reported. Age and initial body weights were not reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were housed individually. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.			
Dennein 2. Selection e	- 1 D						
Domain 2: Selection a	nd Performance Metric 2:	Allocation	Medium	"Dams were weight ranked and assigned to dose groups to minimized differences in means and variance among treatment groups". It is not clear whether this was done randomly, but this description indicates that normalization procedures were performed to balance important variables across groups. The first three males identified from each litter were selected for measuring ex vivo testicular testosterone production.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, hormone levels body weight) or clinical signs.			
Domain 2: Confoundi	va / Variabla Ca	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and appropriate. There were no indications that husbandry conditions were different between the groups. Food and water intake were not reported in an oral gavage study. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which has been linked to developmental and reproductive health problems. This could potentially confound results; although if control animals were exposed to the same levels, this may not substantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plas- tic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophos- phates. No analysis of food for potential endocrine disruptors was conducted.			
D							
Domain 4: Selective R	eporting and At	trition					

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 788239 Table: 4 of 6

			continued from previous p	age			
Study Citation: Health Outcome(s)	 Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Mortality-Mortality (results reported for DINP and DIBP only)-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only). 						
and Reported	DIBP only)	DIBP only)					
Health Effect(s):	Oral Cavage						
Fynosure Route	Ofal-Gavage	e-Duration. Reproductive/Developmental-1-	-r0 - gestation (OD 14-18)				
Snecies:	Rat-Other (S	Sprague-Dawley- Charles River)-Female-Ra	t-Wistar - [rat]-Female				
Chemical:	Diethylhexy	Phthalate- Parent compound	a mistar [rad] remaie				
HERO ID:	788239						
Domain		Metric	Rating	Comments			
Domain	Metric 5	Selective Reporting and Attrition	Low	The methods state that there were 3.6 dams treated/group. It was not specified whether			
	menie 3.		2011	the methods state that there were 5 o dams treated group. It was not spectruled whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from $n = 3$ controls and $n =$ 4 for all other dose groups. However, the sample sizes for T production were from $n = 6, 3, 3, 6, 4, 4,$ and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.			
		.,					
Domain 5: Exposure M	ethods Sensitiv Metric 6:	Ity Chemical administration and characterization	Medium	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.			
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure from GD 14-18 occurs at the end of the critical window of organogenesis and does not include pre-mating or early gestational stages, so may be less sensitive for evaluating maternal effects and effects on fetal survival and growth.			
Domain 6: Outcome M	easures and Re Metric 8:	sults Display Endpoint sensitivity and specificity	Low	The methods did not specify that dams were observed for mortality or clinical signs and no results for these endpoints were reported for animals treated with DEHP; however, results for these endpoints were reported for animals treated with other chemicals (e.g., DINP, DIBP) and therefore, it is assumed that these endpoints were evaluated in all animals in the study. No details were provided on the frequency of observations.			
	Metric 9:	Results presentation	Uninformative	No results for mortality or clinical observations were provided in the text.			
Additional Comments:	None						
Overall Quali	ty Deteri	nination	Uninformative				

Continued on next page ...

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Diethylhexyl Phthalate

HERO ID: 788239 Table: 4 of 6

		continued from previous page			
Study Citation:	Hannas, B. R., Lambright, C. S., Furr, J., Howdesh and gene expression levels in rat testes following phthalate. Toxicological Sciences 123(1):206-216.	nell, K. L., Wilson, V. S., Gray, L. E. (2011 in utero exposure to diethylhexyl phthalato). Dose-response assessment of fetal testosterone production e, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl		
Health Outcome(s)	Mortality-Mortality (results reported for DINP and	DIBP only)-Other (please specify below)	(Clinical signs)-Overt toxicity (results reported for DINP and		
and Reported	DIBP only)				
Health Effect(s):					
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)				
Exposure Route:					
Species:	Rat-Other (Sprague-Dawley- Charles River)-Fema	le-Rat-Wistar - [rat]-Female			
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	788239				
Domain	Metric	Rating	Comments		

Study Citation: Health Outcome(s) and Reported	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain				
Health Effect(s): Duration and Exposure Route: Species:	Oral-Gavage	e-Duration: Reproductive/Developmental-1-F	F0 - gestation (C	GD 14-18) Semale	
Chemical: HERO ID:	Diethylhexy 788239	l Phthalate- Parent compound	- Wistai - [iat]-i		
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance identity and source were reported. Purity or grade were not reported. Test animal species, strain, sex, and source were reported. Age and initial body weights were not reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were housed individually. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.	
Domain 2: Selection and	d Performance Metric 2:	Allocation	Medium	"Dams were weight ranked and assigned to dose groups to minimized differences in means and variance among treatment groups". It is not clear whether this was done randomly, but this description indicates that normalization procedures were performed to balance important variables across groups. The first three males identified from each litter were selected for measuring ex vivo testicular testosterone production	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, hormone levels, body weight) or clinical signs.	
Domain 3: Confounding	r / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and appropriate. There were no indications that husbandry conditions were different between the groups. Food and water intake were not reported in an oral gavage study. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which has been linked to developmental and reproductive health problems. This could potentially confound results; although if control animals were exposed to the same levels, this may not substantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plas- tic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophos- phates. No analysis of food for potential endocrine disruptors was conducted.	
Domain 4: Selective Re	porting and At	trition			

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 788239 Table: 5 of 6

		conti	nued from previ	ious page		
Study Citation: Health Outcome(s) and Reported Health Effect(c):	Hannas, B. and gene ex phthalate. T Reproductiv	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain				
Duration and Exposure Route:	Oral-Gavag	e-Duration: Reproductive/Developmental-1	-F0 - gestation (C	GD 14-18)		
Species: Chemical: HERO ID:	Rat-Other (Diethylhexy 788239	Sprague-Dawley- Charles River)-Female-R yl Phthalate- Parent compound	at-Wistar - [rat]-F	Female		
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from $n = 3$ controls and $n = 4$ for all other dose groups. However, the sample sizes for T production were from $n = 6, 3, 3, 6, 4, 4, and 3$ litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.		
Domain 5: Exposure M	Aethods Sensiti	vity				
Domani 5. Exposure iv	Metric 6:	Chemical administration and characterization	Medium	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.		
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure frequency, timing and duration were appropriate for the study's aim. Preg- nant dams were dosed daily from GD 14-18, which coincides with the critical window of male sexual differentiation (Dent et al. 2015 [3452649]; Scott et al. 2009 [673313]).		
Domain 6: Outcome M	langurag and D	aculta Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	No details are provided on how litter size was calculated and whether it includes both live and dead fetuses. There are also concerns for the sample size; in another publica- tion by this group (Furr et al. 2014 [2510906]), the authors state that n=3 does not have enough statistical power to detect anything other than large changes in fetal survival.; Maternal body weight gain: Authors do not correct for gravid uterine weight or report fetal body weights, so maternal toxicity cannot be distinguished from fetal effects. There are also concerns for the sample size; in another publication by this group (Furr et al. 2014 [2510906]), authors state that this sample size (n=3 dams/dose group) is not ad- equate to consistently detect anything other than rather large alterations of maternal weight gain.		

PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

		•	continued from previo	ous page	
Study Citation:	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216.				
Health Outcome(s) and Reported	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain				
Health Effect(s): Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)				
Exposure Route:					
Species:	Rat-Other (S	Sprague-Dawley- Charles River)-Fe	male-Rat-Wistar - [rat]-F	emale	
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	788239				
Domain		Metric	Rating	Comments	
	Metric 9: Results presentation Medium Body weight data is not reported for all dams (3 control and 4 in treatment group body weight gain is shown graphically. Statistical analysis is performed by the authors. Other data are reported as negative in the text.				
Additional Comments:	None				

Overall Quality Determination

Medium

Study Citation: Health Outcome(s) and Reported Health Effect(s):	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain				
Duration and Exposure Route: Species: Chemical:	Oral-Gavag Rat-Other (S Diethylhexy	e-Duration: Reproductive/Developmental-1-F Sprague-Dawley- Charles River)-Female-Rat l Phthalate- Parent compound	F0 - gestation (C -Wistar - [rat]-F	GD 14-18) Female	
HERO ID:	788239				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance identity and source were reported. Purity or grade were not reported. Test animal species, strain, sex, and source were reported. Age and initial body weights were not reported. Husbandry conditions (temperature, humidity, and light cycle) were reported. Animals were housed individually. Food and water were available ad libitum. The dose levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.	
Domain 2: Selection an	d Performance Metric 2:	Allocation	Medium	"Dams were weight ranked and assigned to dose groups to minimized differences in means and variance among treatment groups". It is not clear whether this was done randomly, but this description indicates that normalization procedures were performed to balance important variables across groups. The first three males identified from each litter were selected for measuring ex vivo testicular testosterone production.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, hormone levels, body weight) or clinical signs.	
Domain 3: Confoundin	g / Variable Co	pptrol			
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and appropriate. There were no indications that husbandry conditions were different between the groups. Food and water intake were not reported in an oral gavage study. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which has been linked to developmental and reproductive health problems. This could potentially confound results; although if control animals were exposed to the same levels, this may not substantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plas- tic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophos- phates. No analysis of food for potential endocrine disruptors was conducted.	
Domain 4: Selective Re	porting and At	trition			
Continued on next page					

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 788239 Table: 6 of 6

		conti	nued from previ	ious page		
Study Citation: Health Outcome(s) and Reported Health Effect(c):	Hannas, B. and gene ex phthalate. T Reproductiv	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216. Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain				
Duration and Exposure Route:	Oral-Gavag	e-Duration: Reproductive/Developmental-1	-F0 - gestation (C	GD 14-18)		
Species: Chemical: HERO ID:	Rat-Other (Diethylhexy 788239	Sprague-Dawley- Charles River)-Female-R yl Phthalate- Parent compound	at-Wistar - [rat]-F	Female		
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from $n = 3$ controls and $n = 4$ for all other dose groups. However, the sample sizes for T production were from $n = 6, 3, 3, 6, 4, 4, and 3$ litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.		
Domain 5: Exposure M	Aethods Sensiti	vity				
Domani 5. Exposure iv	Metric 6:	Chemical administration and characterization	Medium	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.		
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure frequency, timing and duration were appropriate for the study's aim. Preg- nant dams were dosed daily from GD 14-18, which coincides with the critical window of male sexual differentiation (Dent et al. 2015 [3452649]; Scott et al. 2009 [673313]).		
Domain 6: Outcome M	langurag and D	aculta Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	No details are provided on how litter size was calculated and whether it includes both live and dead fetuses. There are also concerns for the sample size; in another publica- tion by this group (Furr et al. 2014 [2510906]), the authors state that n=3 does not have enough statistical power to detect anything other than large changes in fetal survival.; Maternal body weight gain: Authors do not correct for gravid uterine weight or report fetal body weights, so maternal toxicity cannot be distinguished from fetal effects. There are also concerns for the sample size; in another publication by this group (Furr et al. 2014 [2510906]), authors state that this sample size (n=3 dams/dose group) is not ad- equate to consistently detect anything other than rather large alterations of maternal weight gain.		

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Diethylhexyl Phthalate

			continued from previo	bus page	
Study Citation:	Hannas, B. I and gene exp phthalate. To	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicological Sciences 123(1):206-216.			
Health Outcome(s)	Reproductiv	e/Developmental-Developmental	-litter size; fetal mortality-N	Nutritional/Metabolic-Maternal body weight and body weight gain	
and Reported Health Effect(s):					
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)				
Exposure Route:					
Species:	Rat-Other (S	Sprague-Dawley- Charles River)-F	emale-Rat-Wistar - [rat]-Fe	emale	
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	788239				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	Medium	Body weight data is not reported for all dams (3 control and 4 in treatment groups). The body weight gain is shown graphically. Statistical analysis is performed by the study authors. Other data are reported as negative in the text.	
Additional Comments:	None				

Overall Quality Determination

Medium

Study Citation:	Hellwig, J., 35(5):501-51	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). Differential prenatal toxicity of branched phthalate esters in rats. Food and Chemical Toxicology 35(5):501-512.				
Health Outcome(s) and Reported Health Effect(s):	Mortality-Ma	Mortality-Maternal lethality				
Duration and Exposure Route:	Oral-Gavage	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)				
Species:	Rat-Wistar -	[rat]-Female				
Chemical:	Diethylhexyl	Phthalate- Parent compound				
HERO ID:	674193 Link	ed HERO ID(s): 674193, 1325530				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Qu	ıality					
	Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and general compositions were reported. Although purities were measured, they were not reported. Animal species, strain, sex, and source were specified. Age was defined as "sexually mature;" starting body weights were reported. Animals were virgins. No animal husbandry details were included in the current study. However, the study referenced HERO ID 673425 and indicated that the current study was performed as described in the author's previous publication. In the cited reference, husbandry conditions were described (temperature, humidity, light/dark cycle, and food and water availability were specified). The number of animals per cage was not reported. Animals were dosed orally, via gavage from GD 6-15. Limited endpoint evaluation methods along with quantitative results for most endpoints were provided.		
Domain 2: Selection and	l Performance					
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normaliza- tion were provided.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified for any outcome; however, the outcomes were generally not subjective in nature, or were simple measures that do not require a blinded assessment.		
Domain 3: Confounding	/ Variable Cor	ntrol				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehi- cle. A vehicle control may have been more appropriate. There are no concerns regarding the control responses. There were some reductions in food consumption in high-dose animals, but there was no impact on animal body weights. It is unclear why the number of animals per group was inconsistent. No other confounding variables were reported.		
Domain 4: Selective Rer	porting and Att	rition				
	Metric 5:	Selective Reporting and Attrition	High	No animals died. All of the animals are accounted for. There is no evidence of attrition or selective reporting.		

Domain 5: Exposure Methods Sensitivity

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		co	ontinued from previo	us page		
Study Citation:	Hellwig, J., 35(5):501-5	Freudenberger, H., Jäckh, R. (1997). 12.	Differential prenatal	toxicity of branched phthalate esters in rats. Food and Chemical Toxicology		
Health Outcome(s) and Reported	Mortality-M	aternal lethality				
Health Effect(s):						
Duration and	Oral-Gavage	e-Duration: Reproductive/Developmenta	al-1-F0 - gestation (GI	D 6-15)		
Species:	Rat-Wistar -	[rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674193 Link	ted HERO ID(s): 674193, 1325530				
Domain		Metric	Rating	Comments		
	Metric 6:	characterization	Medium	Animals were dosed via gavage and gavage solutions were prepared fresh daily in olive oil (olive oil DAB 9/10); the gavage volume (5 mL/kg) was reported. There was no mention of testing for homogeneity, but the test substance was soluble. Because the solutions were prepared fresh, storage is less likely to be an issue. The test substances were obtained and produced by BASF Aktingesellschaft and were purportedly analyzed for purity by the supplier using gas chromatography. Samples were not analyzed by the performing laboratory and the actual purities were not reported. Specific details of each chemical, specifically details of branching on of the alcohol moieties were provided. The nominal doses were calculated based on animal body weights measured at the be- ginning of the dosing period.		
	Metric 7:	Exposure timing, frequency, and duration	High	The purpose of this study was to evaluate prenatal toxicity, which included evaluations of skeletal malformations. The animals were dosed from GD 6-15 which does not include the sensitive window for skeletal development (e.g., GD 6-19). However, the exposure timing, frequency, and duration were appropriate for other non-developmental outcomes.		
Domain 6: Outcome Me	asures and Re	sults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The authors adequately justified the doses and spacing, which was based on data from other studies. There were no concerns with the test species, but the number of ani- mals/group and sample sizes (7-10 animals/group) were less than recommended by current guidelines for this study type specifying at least 20 pregnant females/group. However, the number of animals was sufficient for this outcome of interest. Animals from all groups were assessed. There are no concerns for endpoint sensitivity and specificity.		
	Metric 9:	Results presentation	High	The data tables included maternal lethality. No animals died and statistical analysis was not necessary.		
Additional Comments:	None					
Overall Qualit	y Deterr	nination	Medium			

Study Citation:	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). 35(5):501-512.	. Differential prenatal toxicity	of branched phthalate esters in rats. Food and Chemical Toxicology
Health Outcome(s)	Reproductive/Developmental-Reproductive: Uteru	is weight, corpora lutia/dam, i	nplantations sites/dam, placental weight; Developmental: pre and post
and Reported	implantation loss, total resorptions, live fetuses, fet	tal weights, fetal and skeletal va	riations and malformations
Health Effect(s):			
Duration and	Oral-Gavage-Duration: Reproductive/Development	tal-1-F0 - gestation (GD 6-15)	
Exposure Route:			
Species:	Rat-Wistar - [rat]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530		
Domain	Metric	Rating	Comments

Domain	Metric	Rating	Comments		
Domain 1: Reporting Quality					
Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and gen- eral compositions were reported. Although purities were measured, they were not re- ported. Animal species, strain, sex, and source were specified. Age was defined as "sex- ually mature;" starting body weights were reported. Animals were virgins. No animal husbandry details were included in the current study. However, the study referenced HERO ID 673425 and indicated that the current study was performed as described in the author's previous publication. In the cited reference, husbandry conditions were de- scribed (temperature, humidity, light/dark cycle, and food and water availability were specified). The number of animals per cage was not reported. Animals were dosed orally, via gavage from GD 6-15. Limited endpoint evaluation methods along with quan- titative results for most endpoints were provided.		
Domain 2: Selection and Performance					
Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normaliza- tion were provided.		
Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified for any outcome; however, the outcomes were generally not subjective in nature, or were simple measures that do not require a blinded assessment.		
Domain 3: Confounding / Variable Co	ntrol				
Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehi- cle. A vehicle control may have been more appropriate. There are no concerns regarding the control responses. There were some reductions in food consumption in high-dose animals. It is unclear whether this was related to palatability, but there was no impact on animal body weights. It is unclear why the number of animals per group was inconsis- tent. No other confounding variables were reported.		
Domain 4: Selective Reporting and At	trition				
Metric 5:	Selective Reporting and Attrition	High	No animals died. All of the animals are accounted for. There is no evidence of attrition or selective reporting.		
Domain 5: Exposure Methods Sensitivity					
Continued on next page					

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Diethylhexyl Phthalate

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Study Citation:	Hellwig, J., Freudenberger, H., Jäckh, R. (1997) 35(5):501-512	7). Differential prenatal toxicity	of branched phthalate esters in rats. Food and Chemical Toxicology
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Reproductive: Ute implantation loss, total resorptions, live fetuses, f	erus weight, corpora lutia/dam, in fetal weights, fetal and skeletal va	nplantations sites/dam, placental weight; Developmental: pre and post riations and malformations
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developme	ental-1-F0 - gestation (GD 6-15)	
Species:	Rat-Wistar - [rat]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530		
Domain	Metric	Pating	Comments

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	Animals were dosed via gavage and gavage solutions were prepared fresh daily in olive oil (olive oil DAB 9/10); the gavage volume (5 mL/kg) was reported. There was no men- tion of testing for homogeneity, but the test substance was soluble. Because the solutions were prepared fresh, storage is less likely to be an issue. The test substances were ob- tained and produced by BASF Aktiengesellschaft and were purportedly analyzed for purity by the supplier using gas chromatography. Samples were not analyzed by the performing laboratory and the actual purities were not reported. Specific details of each chemical, specifically details of branching on of the alcohol moieties were provided. The nominal doses were calculated based on animal body weights measured at the be- ginning of the dosing period.
	Metric 7:	Exposure timing, frequency, and duration	Low	The purpose of this study was to evaluate prenatal toxicity, which included evaluations of skeletal malformations. The animals were dosed from GD 6-15 which does not include the sensitive window for skeletal development (e.g., GD 6-19).
Domain 6: Outcome Me	easures and Re	esults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	The authors adequately justified the doses and spacing, which were based on data from other studies. There were no concerns with the test species; however, the number of animals/group and sample sizes (7-10 animals/group) were less than recommended by current guidelines for this study type (at least 20 pregnant females/group are preferred). Readers are referred to another publication by the same authors for details on the outcome assessment methods (HERO ID 673425). There are no concerns for the outcome assessment methods.
	Metric 9:	Results presentation	Medium	Mean uterine weights and fetal body weights were reported with no measures of vari- ance. Summary incidence data external, visceral, and skeletal changes were sufficient. Statistical analysis was described. It wasn't explicitly stated that the litter was used as the experimental unit, but this is assumed based on the data provided.
Additional Comments:	None			
Overall Quali	ty Deter	mination	Medium	

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Study Citation:	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). 35(5):501-512.	. Differential prenatal toxicity	of branched phthalate esters in rats. Food and Chemical Toxicology
Health Outcome(s) and Reported	Other (please specify below) (Clinical signs)-Mater	rnal clinical signs-Nutritional/M	etabolic-Maternal body weights, food consumption, body weight change
Health Effect(s):			
Duration and	Oral-Gavage-Duration: Reproductive/Developmen	tal-1-F0 - gestation (GD 6-15)	
Exposure Route:			
Species:	Rat-Wistar - [rat]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530		
Domain	Metric	Rating	Comments

Domain 1: Reporting Quality					
Metric 1	: Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and gen- eral compositions were reported. Although purities were measured, they were not re- ported. Animal species, strain, sex, and source were specified. Age was defined as "sex- ually mature;" starting body weights were reported. Animals were virgins. No animal husbandry details were included in the current study. However, the study referenced HERO ID 673425 and indicated that the current study was performed as described in the author's previous publication. In the cited reference, husbandry conditions were de- scribed (temperature, humidity, light/dark cycle, and food and water availability were specified). The number of animals per cage was not reported. Animals were dosed orally, via gavage from GD 6-15. Limited endpoint evaluation methods along with quan- titative results for most endpoints were provided.		
Domain 2: Selection and Performa	nce				
Metric 2	2: Allocation	Low	No details describing the method of animal allocation or other indicators of normaliza- tion were provided.		
Metric 3	B: Observational Bias / Blinding Changes	Medium	Blinding was not specified for any outcome; however, the outcomes were generally not subjective in nature, or were simple measures that do not require a blinded assessment.		
Domain 3: Confounding / Variable	e Control				
Metric 4	E: Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehi- cle. A vehicle control may have been more appropriate. There are no concerns regarding the control responses. There were some reductions in food consumption in high-dose animals, but there was no impact on animal body weights. It is unclear why the number of animals per group was inconsistent. No other confounding variables were reported.		
Domain 4: Selective Reporting and	d Attrition				
Metric 5	S: Selective Reporting and Attrition	High	No animals died. All of the animals are accounted for. There is no evidence of attrition or selective reporting.		
Domain 5: Exposure Methods Sensitivity					
	Contin	ued on next pa	ge		

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Diethylhexyl Phthalate

		co	ntinued from previo	ous page		
Study Citation:	Hellwig, J., 35(5):501-5	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). Differential prenatal toxicity of branched phthalate esters in rats. Food and Chemical Toxicology 35(5):501-512.				
Health Outcome(s) and Reported Health Effect(s):	Other (pleas	Other (please specify below) (Clinical signs)-Maternal clinical signs-Nutritional/Metabolic-Maternal body weights, food consumption, body weight change				
Duration and Exposure Route:	Oral-Gavag	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)				
Species:	Rat-Wistar	Rat-Wistar - [rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	674193 Lin	ked HERO ID(s): 674193, 1325530				
Domain		Metric	Rating	Comments		
	Metric 6:	Chemical administration and characterization	Medium	Animals were dosed via gavage and gavage solutions were prepared fresh daily in olive oil (olive oil DAB 9/10); the gavage volume (5 mL/kg) was reported. There was no men- tion of testing for homogeneity, but the test substance was soluble. Because the solutions were prepared fresh, storage is less likely to be an issue. The test substances were ob- tained and produced by BASF Aktiengesellschaft and were purportedly analyzed for purity by the supplier using gas chromatography. Samples were not analyzed by the performing laboratory and the actual purities were not reported. Specific details of each chemical, specifically details of branching on of the alcohol moieties were provided. The nominal doses were calculated based on animal body weights measured at the be- ginning of the dosing period.		

	Metric 7:	Exposure timing, frequency, and duration	High	The nominal doses were calculated based on animal body weights measured at the be- ginning of the dosing period. The purpose of this study was to evaluate prenatal toxicity, which included evaluations of skeletal malformations. The animals were dosed from GD 6-15 which does not in- clude the sensitive window for skeletal development (e.g., GD 6-19). However, the exposure timing, duration, and frequency were adequate for the selected outcomes of interest.
6: Outcome	Measures and Re Metric 8:	sults Display Endpoint sensitivity and specificity	Medium	The authors adequately justified the doses and spacing, which were based on data from other studies. There were no concerns with the test species; however, the number of

animals/group and sample sizes (7-10 animals/group) were less than recommended by current guidelines for this study type (at least 20 pregnant females/group are preferred). However, the sample size was adequate for the selected outcomes of interest. Another study by the same authors was referenced for the outcome assessment methods (HERO 673425). The protocols were sensitive to the outcomes of interest and consistent with

Body weight data were presented as means without measures of variance. Individual data were not provided. Clinical signs were described in the text for one dose group. Quantitative data for all groups was not provided. Statistic methods were described and

those specified in OECD TG 414.

were appropriate.

Additional Comments: None

Domain

Overall Quality Determination

Metric 9:

Results presentation

Medium

Low

scribed (temperature, humidity, light/dark cycle, and food and water availability were specified). The number of animals per cage was not reported. Animals were dosed

Study Citation:	Hellwig, J., 35(5):501-5	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). Differential prenatal toxicity of branched phthalate esters in rats. Food and Chemical Toxicology 35(5):501-512.					
Health Outcome(s)	Hepatic/Liv	er-Maternal liver weights-Renal/Kidney	y-Maternal kidney we	ights			
and Reported	-						
Health Effect(s):							
Duration and	Oral-Gavag	e-Duration: Reproductive/Development	tal-1-F0 - gestation (C	SD 6-15)			
Exposure Route:	-						
Species:	Rat-Wistar ·	- [rat]-Female					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	674193 Lin	ked HERO ID(s): 674193, 1325530					
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and gen- eral compositions were reported. Although purities were measured, they were not re- ported. Animal species, strain, sex, and source were specified. Age was defined as "sex- ually mature;" starting body weights were reported. Animals were virgins. No animal husbandry details were included in the current study. However, the study referenced HERO ID 673425 and indicated that the current study was performed as described in the author's previous publication. In the cited reference, husbandry conditions were de-			

				orally, via gavage from GD 6-15. Limited endpoint evaluation methods along with quan- titative results for most endpoints were provided.	
Domain 2: Selection and	l Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normaliza- tion were provided.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified for any outcome; however, the outcomes were generally not subjective in nature, or were simple measures that do not require a blinded assessment.	
Domain 3: Confounding	/ Variable Cor	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehi- cle. A vehicle control may have been more appropriate. There are no concerns regarding the control responses. There were some reductions in food consumption in high-dose animals, but there was no impact on animal body weights. It is unclear why the number of animals per group was inconsistent. No other confounding variables were reported.	
Domain 4: Selective Rep	porting and Att Metric 5:	rition Selective Reporting and Attrition	High	No animals died. All of the animals are accounted for. There is no evidence of attrition	
Domain 5: Exposure Me	thods Sensitiv	ity		or selective reporting.	
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Diethylhexyl Phthalate

heptanol-1. The nominal doses were calculated based on animal body weights at the

The purpose of this study was to evaluate prenatal toxicity, which included evaluations

beginning of the dosing period.

			ntinued from previo	ous page	
Study Citation:	Hellwig, J., Freudenberger, H., Jäckh, R. (1997). Differential prenatal toxicity of branched phthalate esters in rats. Food and Chemical Toxicology 35(5):501-512.				
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Maternal liver weights-Renal/Kidney-Maternal kidney weights				
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)				
Species:	Rat-Wistar -	· [rat]-Female			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	674193 Linl	ked HERO ID(s): 674193, 1325530			
Domain		Metric	Rating	Comments	
	Metric 6:	Chemical administration and characterization	Medium	Animals were dosed via gavage and gavage solutions were prepared fresh daily in olive oil; the gavage volume (5 mL/kg) was reported. There was no mention of testing for homogeneity, but the test substance was soluble. Because the solutions were prepared fresh, storage is less likely to be an issue. The test substance was reported to be of commercial origin, but the exact source was not specified. The purity was ≥99%. The alcohol moiety consisted of equivalent amounts of 3,4-, 4,6-, 3,5-, 4,5-, and 5,6-dimethyl-	

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		duration		of skeletal malformations. The animals were dosed from GD 6-15 which does not in- clude the sensitive window for skeletal development (e.g., GD 6-19). However, the exposure timing, duration, and frequency were adequate for the selected outcomes of interest.
Domain 6: Outcome Me	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	The authors adequately justified the doses and spacing, which were based on data from other studies. There were no concerns with the test species; however, the number of animals/group and sample sizes (7-10 animals/group) were less than recommended by current guidelines for this study type (at least 20 pregnant females/group are preferred). Another study by the same authors was referenced for the outcome assessment methods (HERO 673425). The protocols were only partially sensitive to the outcomes of interest; organ weights were measured in the absence of supporting clinical chemistry and microscopic analysis.
	Metric 9:	Results presentation	Low	Organ weight data were presented as means without measures of variance. Statistical methods were described and were appropriate. In some instances only relative, but not absolute organ weights were reported.
Additional Comments:	None			

High

Overall Quality Determination

Metric 7:

Exposure timing, frequency, and

Medium

Study Citation: Health Outcome(s) and Reported	Howdeshell, K. L., Wilson, V. S., Furr, J., Lambright, C. R., Rider, C. V., Blystone, C. R., Hotchkiss, A. K., Gray, L. E., Jr (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicological Sciences 105(1):153-165. Reproductive/Developmental-Male reproductive - testosterone							
Duration and Exposure Route:	Oral-Gavage	-Duration: Reproductive/Developmental-1-	-F0 - gestatio	on (GD 8-18)				
Species:	Rat-Sprague	Rat-Sprague-Dawley - [rat]-Both						
HERO ID:	675206	Prinalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Reporting Qu	aality Metric 1:	Reporting Quality	High	Good. All critical and most important information was reported. Reported informa- tion included information on the test substance (name, source, purity), the test model (species, strain, sex, and source, animal husbandry details (animals per cage, photope- riod, temperature, food and water availability), exposure methods, experimental design, endpoint evaluations, and presentation of results. Missing information included the test animal age, initial body weights, parity, and humidity.				
Domain 2: Selection and	Performance							
Domani 2. Selection and	Metric 2:	Allocation	Medium	Adequate. Authors stated pregnant dams were assigned to treatment groups on GD 8 in a manner that provided similar mean body weight per treatment group prior to dosing. It is not clear whether this was done randomly, but this description indicates that normal- ization procedures were performed to balance important variables across groups.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	All outcomes: Adequate. The paper did not indicate that whether investigators were blinded during outcome assessment. However, via personal correspondence, authors indicated that fetal dissections were performed by investigators that were unaware of the treatment group. Potential concern for bias was mitigated because all outcomes reported in this study are relatively objective measurements.				
Domain 3: Confounding	/ Variable Co	atrol						
	Metric 4:	Confounding / Variable Control	High	Good. Vehicle (laboratory-grade corn oil) and gavage volume were the same in control and treatment groups. Animals were housed individually. The study did not specify whether measures were taken to reduce the potential for exposure to plasticizers, which could influence study results in a study focused on assessing the potential for endocrine disruption. Water was tested monthly for Pseudomonas and every 4 months for a suite of chemicals including pesticides and heavy metals. However, the materials used to dispense water to animals was not specified and it was not reported whether food was tested for phthalate contamination. Animals were housed in polycarbonate rather than metal cages. The experimental conditions described provided no indication of different practices across treatment groups.				

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Study Citation:	Howdeshell phthalate es 105(1):153-	Howdeshell, K. L., Wilson, V. S., Furr, J., Lambright, C. R., Rider, C. V., Blystone, C. R., Hotchkiss, A. K., Gray, L. E., Jr (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicological Sciences 105(1):153-165.						
Health Outcome(s) and Reported	Reproductiv	Reproductive/Developmental-Male reproductive - testosterone						
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-1	-F0 - gestatio	on (GD 8-18)				
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 675206	-Dawley - [rat]-Both l Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
	Metric 5:	Selective Reporting and Attrition	Medium	Adequate. All endpoints described in methods were reported qualitatively or quantita- tively. All dams/litters are accounted for in the maternal weight gain, litter size, resorp- tions, and fetal mortality data (Table 2). A small number of dams died or were removed from the study due to dosing errors, as described in the text. The numbers of fetuses and litters used to determine testicular testosterone production (Table 6) were reported.				
Domain 5: Exposure M	ethods Sensitiv	vity						
Domain 5. Exposure M	Metric 6:	Chemical administration and characterization	Medium	Adequate. Source of chemical was reported (Sigma-Aldrich, who reported a purity of 99%). There was no indication that the authors independently verified the concentration or stability of the test chemical. The vehicle (laboratory grade corn oil) was also purchased from Sigma-Aldrich. Rat dams were weighed daily during the dosing period to administer the dose per kg body weight.				
	Metric 7:	Exposure timing, frequency, and duration	High	All outcomes: Good. Pregnant dams were dosed daily with DIBP from GD 8-18. This exposure covers the period of post-implantation embryonic development, including the critical windows of organogenesis and male sexual differentiation.				
Domain 6: Outcome Me	easures and Re	sults Display						
	Metric 8:	Endpoint sensitivity and specificity	High	Good. There are no concerns regarding the specificity and validity of the protocols and measures were identified. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. Testes from all of the males were incubated for fetal testicular hormone production. The methods stated that both testes were dissected and incubated individually. Results were obtained from the following sample sizes per dose (fetuses, litters): 0 (24, 4), 100 (26, 4), 300 (27, 4), 600 (29, 4), and 900 (20, 4) mg/kg-day. These sample sizes are considered to be adequate.				
	Metric 9:	Results presentation	High	All outcomes: Good. There are no notable concerns about the way the results are analyzed or presented.				
Additional Comments:	Only fetal te	estosterone was evaluated for data quality.						
Overall Quality Determination			High					

Study Citation: Health Outcome(s) and Reported Health Effect(s):	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269. Reproductive/Developmental-Organ weight (testis, epididymis, prostate, seminal vesicles, ovaries including the oviducts, uterus); Histopathology (testis, epididymis, prostate, seminal vesicles, ovaries, organise, concentration and percentage of abnormal sperm); Mating and fertility indices (copulatory plug, number of fertile pairs/number cohabitated, litter/pair); F1: live pup body weight, sex ratio, proportion of pups horn alive number of live pups/litter					
Duration and	Oral-Diet-D	uration: Reproductive/Developme	ntal-1-F0- premating	(7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating		
Exposure Route:	(98 days)					
Species:	Mouse-CD-1	l - [mouse]-Both				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	61566					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Qu	uality					
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and pu- rity (>99%) were reported. Test animal species, strain, sex, age, and source were re- ported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of		

Domain 2: Selection and Pe	erformance			
Ν	fetric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body
				weights.
N	Ietric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in
				nature or consisted of clinical signs or initial histopathology.

Low

tive data.

Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire

cages. Food and water dispensing containers were not described.

Domain 3: Confounding	, / Variable Co	ntrol
	Metric 4:	Confounding

ric 4:	Confounding /	Variable	Control
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Domain 4: Selective Reporting and Attrition

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Diethylhexyl Phthalate

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Study Citation:	Lamb, J., C Pharmacolo	hapin, R., Teague, J., Lawton, A., Reel, gy 88(2):255-269.	J. (1987). Rej	productive effects of four phthalic acid esters in the mouse. Toxicology and Applied
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testis, epididymis, prostate, seminal vesicles, ovaries including the oviducts, uterus); Histopathology (testis, epididymis, prostate, seminal vesicles, ovary, oviduct, uterus, and vagina);Sperm parameters (percent of motile sperm, concentration and percentage of abnormal sperm);Mating and fertility indices (copulatory plug, number of fertile pairs/number cohabitated, litter/pair);F1: live pup body weight, sex ratio, properties of puge born alive, number of live puge/litter			
Duration and	Oral-Diet-D	Duration: Reproductive/Developmental-1-	F0- premating	(7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating
Exposure Route:	(98 days)			
Species:	Mouse-CD-	1 - [mouse]-Both		
Chemical:	Diethylhexy	l Phthalate- Parent compound		
HERO ID:	61566			
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure M	lethods Sensitiv Metric 6: Metric 7:	vity Chemical administration and characterization Exposure timing, frequency, and duration	Low	The source of the test substance was reported. The purity of the test substance was in- dependently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg. Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on pre- vious toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.

Domain 6: Outcome Measures and Results Display

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Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269. Reproductive/Developmental-Organ weight (testis, epididymis, prostate, seminal vesicles, ovaries including the oviducts, uterus); Histopathology (testis, epididymis, prostate, seminal vesicles, ovary, oviduct, uterus, and vagina);Sperm parameters (percent of motile sperm, concentration and percentage of abnormal sperm);Mating and fertility indices (copulatory plug, number of fertile pairs/number cohabitated, litter/pair);F1: live pup body weight, sex ratio, proportion of pups born alive, number of live pups/litter Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566			
Domain	Metric 8: Metric 9:	Metric Endpoint sensitivity and specificity Results presentation	Rating Medium Low	Comments No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, ex- cessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group. Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported. The study used the pup instead of the
Additional Comments: Overall Qualit	None	nination	Low	litter as the unit of statistical analysis, this has the potential to overestimate statistical significance of experimental findings (Dishaw et al. 2020). Individual animal data was not reported.

Overall Quality Determination

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied				
Health Outcome(s) and Reported	Pharmacology 88(2):255-269. Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.	
Domain 3: Confounding	g / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.	

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May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

		cont	tinued from previ	ious page	
Study Citation: Health Outcome(s)	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269. Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:					
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.	
Domain 5. Expansion	lathada Sanaitir				
Domain 5: Exposure iv	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was in- dependently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.	
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on pre- vious toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.	
	r 15				
Domain 6: Outcome M	easures and Re Metric 8:	suits Display Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.	
		Con	tinued on next pa	age	

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HERO ID: 61566 Table: 2 of 8

Diethylhexyl Phthalate	

		continued from previo	bus page		
Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Pharmacology 88(2):255-269.	Reel, J. (1987). Reprodu	ctive effects of four phthalic acid esters in the mouse. Toxicology and Applied		
Health Outcome(s)	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight				
and Reported Health Effect(s):					
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating				
Exposure Route:	(98 days)				
Species:	Mouse-CD-1 - [mouse]-Both				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	61566				
Domain	Metric	Rating	Comments		
	Metric 9: Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.		
	None				

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied				
Health Outcome(s) and Reported	Pharmacology 88(2):255-269. Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.	
Domain 3: Confounding	g / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.	

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Diethylhexyl Phthalate

		cont	tinued from previ	ious page	
Study Citation: Health Outcome(s)	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269. Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:					
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.	
Domain 5. Expansion	lathada Sanaitir				
Domain 5: Exposure iv	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was in- dependently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.	
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on pre- vious toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.	
	r 15				
Domain 6: Outcome M	easures and Re Metric 8:	suits Display Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.	
		Con	tinued on next pa	age	

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HERO ID: 61566 Table: 3 of 8

	,	continued from previo	us page			
Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.					
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight					
Duration and	Oral-Diet-Duration: Reproductive/Developmer	ntal-1-F0- premating (7 day	ys)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating			
Exposure Route:	(98 days)					
Species:	Mouse-CD-1 - [mouse]-Both					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	61566					
Domain	Metric	Rating	Comments			
	Metric 9: Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.			
Additional Comments:	None					
Overall Qualit	ty Determination	Medium				

PUBLIC RELEASE DRAFT

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied				
Health Outcome(s) and Reported	Pharmacology 88(2):255-269. Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:					
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.	
Domain 3: Confounding	g / Variable Co	ntrol			
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.	

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Diethylhexyl Phthalate

in the mouse. Toxicology and Applied w) (Endocrine)-Pituitary weight				
Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
ments				
bular form or within text. Not all animals in Table 12 data for 35-36 control males or 14-15 treated females are reported (out data from these animals were not included. sex ratio but results were not reported for trition are not explained but they are not tation of the results. Sample size was stated				
d. The purity of the test substance was in- d spectrometric analysis to be 99%. Test ely every week, stored at 4°C until used, and Institute for referee analysis. The compound or 7 days. Target test concentrations were ovided. Feed intake and body weights were because pairs were cohabiting that it was e dose in mg/kg.				
temic toxicity at the highest dose and lesser ver, it was unclear if this was based on pre- ed for a 7-day premating period, paired, and ere then separated and exposed for 21 days. s. Groups were treated concurrently. The time similar endpoints suggest a premating s shortened pre-mating period may not have oestrous cycles to sufficiently detect adverse or the maturing of spermatozoa.				
animals/group was appropriate as group pregnant females/group and sufficient for 1 one control group were utilized which was end using doses that are two to four-fold thors used doses with ten-fold intervals, ex- st dose and adverse effects were not observed were reported and considered sensitive for gy were performed only on the highest dose				
animals/ pregnant d one cor end using hors used st dose an were rep gy were p				

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 61566 Table: 4 of 8

Diethylhexyl Phthalate	

		••	. continued from previ	ous page	
Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.				
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight				
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating				
Exposure Route:	(98 days)				
Species:	Mouse-CD-	-1 - [mouse]-Both			
Chemical:	Diethylhexy	yl Phthalate- Parent compound			
HERO ID:	61566				
Domain		Metric	Rating	Comments	
	Metric 9:	Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.	
Additional Comments:	None				
Overall Ouali	tv Deter	mination	Medium		

Study Citation:	Lamb, J., Cl	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied				
Health Outcome(s) and Reported	Pharmacology 88(2):255-269. Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566					
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:						
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance		TT' 1			
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.		
Domain 3: Confounding	g / Variable Cor	ntrol				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.		

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Diethylhexyl Phthalate

in the mouse. Toxicology and Applied w) (Endocrine)-Pituitary weight				
Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
ments				
bular form or within text. Not all animals in Table 12 data for 35-36 control males or 14-15 treated females are reported (out data from these animals were not included. sex ratio but results were not reported for trition are not explained but they are not tation of the results. Sample size was stated				
d. The purity of the test substance was in- d spectrometric analysis to be 99%. Test ely every week, stored at 4°C until used, and Institute for referee analysis. The compound or 7 days. Target test concentrations were ovided. Feed intake and body weights were because pairs were cohabiting that it was e dose in mg/kg.				
temic toxicity at the highest dose and lesser ver, it was unclear if this was based on pre- ed for a 7-day premating period, paired, and ere then separated and exposed for 21 days. s. Groups were treated concurrently. The time similar endpoints suggest a premating s shortened pre-mating period may not have oestrous cycles to sufficiently detect adverse or the maturing of spermatozoa.				
animals/group was appropriate as group pregnant females/group and sufficient for 1 one control group were utilized which was end using doses that are two to four-fold thors used doses with ten-fold intervals, ex- st dose and adverse effects were not observed were reported and considered sensitive for gy were performed only on the highest dose				
animals/ pregnant d one cor end using hors used st dose an were rep gy were p				

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HERO ID: 61566 Table: 5 of 8

		continued from prev	ous page		
Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269				
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight				
Duration and	Oral-Diet-Duration: Reproductive/D	evelopmental-1-F0- premating (7 d	ays)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating		
Exposure Route:	(98 days)				
Species:	Mouse-CD-1 - [mouse]-Both				
Chemical:	Diethylhexyl Phthalate- Parent comp	ound			
HERO ID:	61566				
Domain	Metric	Rating	Comments		
	Metric 9: Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.		
Additional Comments:	None				
Overall Ouali	tv Determination	Medium			

Study Citation:	Lamb, J., C	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied				
Health Outcome(s) and Reported	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity					
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-D (98 days) Mouse-CD- Diethylhexy 61566	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body		
	Metric 3:	Observational Bias / Blinding Changes	Medium	weights. Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.		
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might im- pact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.		

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May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

		conti	inued from previ	ious page	
Study Citation:	Lamb, J., C	Chapin, R., Teague, J., Lawton, A., Reel, J.	. (1987). Reprod	uctive effects of four phthalic acid esters in the mouse. Toxicology and Applied	
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity				
Duration and Exposure Route: Species: Chemical:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylbeyyl Phthelate- Parent compound				
HERO ID:	61566	I I I I I I I I I I I I I I I I I I I			
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.	
Domain 5: Exposure N	lethods Sensitiv	vity			
Domain of Exposure i	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was in- dependently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.	
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on pre- vious toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.	
	r 10				
Domain 6: Outcome M	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies for assessing clinical signs were not reported (frequency, detailed or cage-side observations).	
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Diethylhexyl Phthalate

			continued from previo	us page			
Study Citation:	Lamb, J., C Pharmacolo	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.					
Health Outcome(s)	Nutritional/	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity					
and Reported							
Health Effect(s):							
Duration and	Oral-Diet-D	uration: Reproductive/Development	tal-1-F0- premating (7 da	ys)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating			
Exposure Route:	(98 days)						
Species:	Mouse-CD-	1 - [mouse]-Both					
Chemical:	Diethylhexy	l Phthalate- Parent compound					
HERO ID:	61566						
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	Medium	Clinical signs were reported as negative in the text.			
Additional Comments:	None						
Overall Quali	ty Deteri	nination	Medium				

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Study Citation:	Lamb, J., C	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied				
Health Outcome(s) and Reported	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity					
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-D (98 days) Mouse-CD- Diethylhexy 61566	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.		
Domain 2: Selection and	d Performance Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body		
	Metric 3:	Observational Bias / Blinding Changes	Medium	weights. Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.		
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might im- pact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.		

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Diethylhexyl Phthalate

		cont	inued from previ	ious page
Study Citation:	Lamb, J., C Pharmacolo	hapin, R., Teague, J., Lawton, A., Reel, J 98 88(2):255-269	. (1987). Reprod	uctive effects of four phthalic acid esters in the mouse. Toxicology and Applied
Health Outcome(s) and Reported Health Effect(s):	Nutritional/	Metabolic-Body weight and food intake-Ot	ther (please specif	y below) (Clinical signs)-Clinical signs of toxicity
Duration and	Oral-Diet-D	ouration: Reproductive/Developmental-1-F	0- premating (7 d	ays)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating
Exposure Route:	(98 days)	1 [manual Dath		
Species: Chemical:	Mouse-CD- Diethylhexy	1 - [mouse]-Both /] Phthalate- Parent compound		
HERO ID:	61566			
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure N	lethods Sensitiv	vity		
Domain 5. Exposure iv	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was in- dependently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on pre- vious toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
	1.0			
Domain 6: Outcome M	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies for assessing clinical signs were not reported (frequency, detailed or cage-side observations).
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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 61566 Table: 7 of 8

		•	continued from previo	bus page
Study Citation:	Lamb, J., C	hapin, R., Teague, J., Lawton, A., I	Reel, J. (1987). Reprodu	active effects of four phthalic acid esters in the mouse. Toxicology and Applied
	Pharmacolo	gy 88(2):255-269.		
Health Outcome(s)	Nutritional/	Metabolic-Body weight and food int	take-Other (please specify	y below) (Clinical signs)-Clinical signs of toxicity
and Reported				
Health Effect(s):				
Duration and	Oral-Diet-D	Ouration: Reproductive/Development	tal-1-F0- premating (7 da	ys)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating
Exposure Route:	(98 days)			
Species:	Mouse-CD-	1 - [mouse]-Both		
Chemical:	Diethylhexy	l Phthalate- Parent compound		
HERO ID:	61566	-		
Domain		Metric	Rating	Comments
	Metric 9:	Results presentation	Medium	Clinical signs were reported as negative in the text.
Additional Comments:	None			

Overall Quality Determination

Medium

Study Citation:	Lamb, J., C	hapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reprodu	active effects of four phthalic acid esters in the mouse. Toxicology and Applied
Health Outcome(s) and Reported	Pharmacolo Nutritional/	gy 88(2):255-269. Metabolic-Body weight and food intake		
Health Effect(s): Duration and Exposure Route:	Oral-Diet-D (98 days) Mouse-CD-	uration: Reproductive/Developmental-1-F0-	premating (7 da	ays)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating
Chemical:	Diethylhexy	Philade-Parent compound		
HERO ID:	61566	-		
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection an	d Performance Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body
	Metric 3:	Observational Bias / Blinding Changes	Medium	weights. Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plas- ticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might im- pact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Re	porting and At	trition		

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Diethylhexyl Phthalate

Lamb, J., C	hanin D. Taagua I. Lawston A. D1 I.		
Pharmacolog	napin, K., Teague, J., Lawton, A., Reel, J. gy 88(2):255-269.	(1987). Reprod	uctive effects of four phthalic acid esters in the mouse. Toxicology and Applied
Nutritional/I	Metabolic-Body weight and food intake		
Oral-Diet-D (98 days)	uration: Reproductive/Developmental-1-F)- premating (7 d	ays)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating
Mouse-CD-	1 - [mouse]-Both		
61566	I Phinalate- Parent compound		
	Metric	Rating	Comments
Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
ethods Sensitiv	zity		
Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was in- dependently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on pre- vious toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
1 D			
easures and Re Metric 8:	sults Display Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were not adequately reported. Timing of measurements for food intake and body weights were not reported.
	Oral-Diet-D (98 days) Mouse-CD- Diethylhexy 61566 ethods Sensitiv Metric 5: Metric 6: Metric 7: easures and Re Metric 8:	Nutritional/Metabolic-Body Weight and rood intake Oral-Diet-Duration: Reproductive/Developmental-1-FG (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566 Metric Metric 5: Selective Reporting and Attrition ethods Sensitivity Metric 6: Chemical administration and characterization Metric 7: Exposure timing, frequency, and duration easures and Results Display Metric 8: Endpoint sensitivity and specificity	Nutritional/Metabolic-Body weight and rood make Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 d (98 days) Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 61566 Metric Rating Metric 5: Selective Reporting and Attrition Metric 6: Chemical administration and characterization Metric 7: Exposure timing, frequency, and duration Metric 7: Exposure timing, frequency, and duration easures and Results Display Metric 8: Endpoint sensitivity and specificity Metric 8: Endpoint sensitivity and specificity Medium

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Diethylhexyl Phthalate

		••	. continued from pre	vious page
Study Citation:	Lamb, J., Chap Pharmacology	pin, R., Teague, J., Lawton, A., I 88(2):255-269.	Reel, J. (1987). Repro	ductive effects of four phthalic acid esters in the mouse. Toxicology and Applied
Health Outcome(s)	Nutritional/Me	tabolic-Body weight and food int	ake	
and Reported				
Duration and	Oral-Diet-Dura	ation: Reproductive/Development	al-1-F0- premating (7	days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating
Exposure Route:	(98 days)	1 1	1 2 (
Species:	Mouse-CD-1 -	[mouse]-Both		
Chemical:	Diethylhexyl P	hthalate- Parent compound		
HERO ID:	61566			
Domain		Metric	Rating	Comments
	Metric 9:	Results presentation	Medium	Necropsy body weights were reported as means +/- SE for control and high dose groups. Statistical analysis was reported and appropriate. Body weights at 1 and 13 weeks are not fully reported (SE not included, only males reported, only high-dose and control group reported). Food intake was reported in text as a range for all groups combined.
Additional Comments:	None			

Overall Quality Determination

Medium

Study Citation:	Lin, H., Ge, fetal Leydig	R., Chen, G., Hu, G., Dong, L., Lian, Q cell aggregation after exposure to phthala)., Hardy, D ite in utero.	., Sottas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in Proceedings of the National Academy of Sciences of the United States of America
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route:	105(20):7218 Reproductive analysis; feta Oral-Gavage	3-7222. /Developmental-Birth rates; number of pup l Leydig cell numbers, size, and distributio -Duration: Reproductive/Developmental-1-	os per dam; s on; testicular -F0 - gestatio	ex ratio; male pup body weights (GD21); AGD (male pups); fetal testicular testosterone gene expression; Leydig cell steroidogenic enzyme levels; testis weights on (GD2-20)
Species:	Rat-Long-Ev	ans - [rat]-Female		
Chemical: HERO ID:	Diethylhexyl 698185	Phthalate- Parent compound		
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Low	All critical and some important information was reported. Reported information in- cluded information on the test substance (name and source), the test model (species, strain, sex, and source), exposure methods, experimental design, endpoint evaluations, and presentation of results. Missing information included the purity of the test substance, test animal age, initial body weights, parity, and all animal husbandry details.
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	No details on the allocation of dams into study groups or on the selection of pups for
	Metric 3:	Observational Bias / Blinding Changes	Medium	outcome analysis were provided. Blinding was not specified but the outcomes were simple measures or were measured or quantified using standard laboratory kits.
Domain 2: Confounding	Variable Con	trol		
	Metric 4:	Confounding / Variable Control	Low	A negative corn oil control group was included. There were no differences in dam or pup body weights and gavage volumes were consistent across groups. Consistency of other potentially confounding factors (e.g., animal husbandry conditions) was not re- ported. It is unclear whether the study took measures to minimize the exposure to other plasticizers which could influence the study results for this health outcome.
Domain 4: Selective Re	porting and Att	rition		
	Metric 5:	Selective Reporting and Attrition	Medium	All dams were accounted for in the study and data for the endpoint of interest were reported. However, there is a range of sample sizes for each endpoint that are not justified by the authors, and/or the sample sizes for the endpoints of interest are confusing (see Metric 6.1). It is unclear if data from any animals were excluded.
Domain 5: Exposure Mo	ethods Sensitivi	ty		
		Conti	nued on nex	t page

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 698185 Table: 1 of 2

		cont	inued from p	previous page
Study Citation:	Lin, H., Ge fetal Leydig 105(20):721	, R., Chen, G., Hu, G., Dong, L., Lian, cell aggregation after exposure to phtha 8-7222.	Q., Hardy, D late in utero.	D., Sottas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in Proceedings of the National Academy of Sciences of the United States of America
Health Outcome(s) and Reported Health Effect(s):	Reproductiv analysis; fet	e/Developmental-Birth rates; number of pu al Leydig cell numbers, size, and distributi	ups per dam; s ion; testicular	sex ratio; male pup body weights (GD21); AGD (male pups); fetal testicular testosterone gene expression; Leydig cell steroidogenic enzyme levels; testis weights
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-	1-F0 - gestati	on (GD2-20)
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 698185	vans - [rat]-Female 1 Phthalate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma) was reported, but the purity was not specified. No certificate of analysis was provided, and there is no indication that the test substance was verified by the performing laboratory. Animals were dosed via gavage in corn oil and the gavage volume (1mL/kg) was appropriate. No details on the preparation, storage, or stability of the test solutions were provided. Doses were reported in mg/kg-day. It is not specified whether doses were adjusted daily based on measured body weights. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD2-20. This exposure covers the period of pre- implantation embryonic development, and the critical windows of organogenesis and male sexual differentiation.
Domain 6: Outcome Me	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	Limited methodological details were provided. Testicular steroids were extracted from "the testes of control and DEHP-exposed pups (n = 10 control; n = 12 DEHP)" on GD21 and testosterone concentrations were measured using an ELISA assay. It was not specified whether this was the number of pups per litter, or if one or both testes were evaluated. The number of animals (dams) per group were n = 6 for controls and low and mid-dose groups, and n = 9 for the high dose. No additional methodological details were provided. Two studies (Akingbemi et al., 2001 and 2004) were cited for additional methodological details and were reviewed for this evaluation; these studies did not provide many useful relevant methodological details as they also cited other studies for methodological details. The sample sizes for other endpoints were also a range (e.g., 10-11, or 8-10), but were sufficient for conducting statistical analysis. Some methodological details for gene expression analysis were provided in supporting information files. There are no concerns for the test animals selected. There was no clear justification for the dose selection and spacing, although the doses had been used in previous studies. A NOAEL could not be determined; however, the purpose of the study was more to characterize and understand the mechanisms of already known effects, rather than to identify a NOAEL.
	Metric 9:	Results presentation	Low	Quantitative data were provided for all of the specified endpoints as incidences or as means \pm SEM across all dose groups. Some statistical methods were described, but there is no indication that the litter was used as the experimental unit for any endpoint. Statistical significance is clearly shown. Individual animal data were not provided.

Additional Comments: only fetal testosterone was evaluated for data quality.

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		continued from previous page	
Study Citation:	Lin, H., Ge, R., Chen, G., Hu, G., Dong, fetal Leydig cell aggregation after exposur 105(20):7218-7222.	L., Lian, Q., Hardy, D., Sottas, C., e to phthalate in utero. Proceedings	Li, X., Hardy, M. (2008). Involvement of testicular growth factors in of the National Academy of Sciences of the United States of America
Health Outcome(s)	Reproductive/Developmental-Birth rates; nu	imber of pups per dam; sex ratio; male	pup body weights (GD21); AGD (male pups); fetal testicular testosterone
and Reported	analysis; fetal Leydig cell numbers, size, an	d distribution; testicular gene expressi	on; Leydig cell steroidogenic enzyme levels; testis weights
Health Effect(s):			
Duration and	Oral-Gavage-Duration: Reproductive/Devel	opmental-1-F0 - gestation (GD2-20)	
Exposure Route:			
Species:	Rat-Long-Evans - [rat]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	698185		
Domain	Metric	Rating	Comments
Overall Qual	ity Determination	Low	

Study Citation:	Lin, H., Ge fetal Leydig 105(20):721	, R., Chen, G., Hu, G., Dong, L., Lian, Q. g cell aggregation after exposure to phthalate 8-7222.	, Hardy, D., So e in utero. Pro	ottas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in ceedings of the National Academy of Sciences of the United States of America
Health Outcome(s) and Reported Health Effect(s):	Nutritional/	Metabolic-Dam body weights		
Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-1-F	-0 - gestation (C	3D2-20)
Species:	Rat-Long-E	vans - [rat]-Female		
Chemical:	Diethylhexy	l Phthalate- Parent compound		
HERO ID:	698185			
Domain	1.	Metric	Rating	Comments
Domain 1: Reporting Q	Metric 1:	Reporting Quality	Low	All critical and some important information was reported. Reported information in- cluded information on the test substance (name and source), the test model (species, strain, sex, and source), exposure methods, experimental design, endpoint evaluations, and presentation of results. Missing information included the purity of the test substance, test animal age, initial body weights, parity, and all animal husbandry details.
Domain 2: Selection an	nd Performance			
Domain 2. Selection an	Metric 2:	Allocation	Low	No details on the allocation of dams into study groups were provided.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcomes were simple measures (dam body weights)
Domain 3: Confoundin	g / Variable Co	antrol		
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. There were no differences in dam body weights and gavage volumes were consistent across groups. Consistency of other potentially confounding factors (e.g., animal husbandry conditions) was not reported. It is unclear whether the study took measures to minimize the exposure to other plasticizers.
Domain 4: Salactiva Pa	porting and At	trition		
	Metric 5:	Selective Reporting and Attrition	High	All dams were accounted for in the study and data for the endpoint of interest were reported.
Domain 5: Exposure M	lethods Sensitiv	vity		
Lonian 5. Exposure M	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma) was reported, but the purity was not specified. No certificate of analysis was provided, and there is no indication that the test substance was verified by the performing laboratory. Animals were dosed via gavage in corn oil and the gavage volume (1mL/kg) was appropriate. No details on the preparation, storage, or stability of the test solutions were provided. Doses were reported in mg/kg-day. It is not specified whether doses were adjusted daily based on measured body weights. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD2-20. This exposure covers the period of pre- implantation embryonic development, and the critical windows of organogenesis and male sexual differentiation, which was the focus of the study.
Domain 6: Outcome M	easures and Re	esults Display		
		Contin	ued on next pa	nge

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Diethylhexyl Phthalate

		conti	inued from previ	ous page
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Lin, H., Ge fetal Leydig 105(20):721 Nutritional/	e, R., Chen, G., Hu, G., Dong, L., Lian, G g cell aggregation after exposure to phthal 8-7222. Metabolic-Dam body weights	Q., Hardy, D., So ate in utero. Proc	ttas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in ceedings of the National Academy of Sciences of the United States of America
Duration and Exposure Route:	Oral-Gavag	e-Duration: Reproductive/Developmental-1	-F0 - gestation (C	GD2-20)
Species: Chemical: HERO ID:	Rat-Long-E Diethylhexy 698185	vans - [rat]-Female /l Phthalate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	Limited methodological details were provided. The data figure legend indicates that dams were weighed before and after exposure, but the exact timing of the measurements was not specified. This is not expected to have a significant impact on the study results. The sample sizes were specified (all animals were weighed). There are no concerns over the test animals selected for the study. A wide dose range was used for this study. There was no clear justification for the dose selection and spacing, although the doses had been used in previous studies.
	Metric 9:	Results presentation	High	Quantitative data were provided as means \pm SEM across all dose groups. Statistical methods were described and were appropriate. Individual animal data were not provided.
Additional Comments:	only fetal te	stosterone was evaluated for data quality.		

Overall Quality Determination

Medium

Study Citation:	Lin, H., Lia	n, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D	., Chen, G.	, Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to
Health Outcome(s) and Reported Health Effect(s):	diethylhexyl Nutritional/N	-phthalate affect two populations of Leydig Aetabolic-Maternal body weights (GD 12 at	cells in mal nd GD 20 ar	e Long-Evans rats. Biology of Reproduction 80(5):882-888. ad GD 21.5)
Duration and Exposure Route:	Oral-Gavage	-Duration: Reproductive/Developmental-1-	F0 - gestatio	on (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)
Species:	Rat-Long-Ev	ans - [rat]-Female		
Chemical: HERO ID:	Diethylhexyl	Phthalate- Parent compound		
Domain	071101	Metric	Rating	Comments
Domain 1: Reporting Qu	uality		Tuning	
	Metric 1:	Reporting Quality	Low	All critical information is reported. Test substance is identified by name, and the sup- plier is reported. The test substance purity was not reported. Test animal species, strain, sex, and commercial source were reported. Starting body weights are reported in the results (table 1). Test animal starting age was not reported but described as "adult" and animal husbandry conditions were not described in any detail, with the authors merely stating that "all animal procedures were performed in accordance with the policies of the Rockefeller University's Animal Care and Use Committee". These deficiencies could significantly impact the quality of the results.
Domain 2: Selection and	1 Performance			
	Metric 2:	Allocation	Low	The authors did not explain how animals were allocated into groups.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were described, but endpoints of interest are unlikely to be significantly influenced by observational bias as they were not subjective.
Domain 3: Confounding	g / Variable Cor	ntrol		
	Metric 4:	Confounding / Variable Control	Medium	An appropriate negative vehicle control is included and there was no response in the control group. Not all information was reported to evaluate confounding (food/water consumption, presence of EDCs in bedding materials). The authors did not discuss whether measures were taken to reduce exposure to plasticizers but this is not expected to significantly impact the endpoint described.
Domain 4: Selective Rep	porting and Att	rition		
		Conti	nued on nex	at page

Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697737 Table: 1 of 2

n, H., Liai ethylhexyl atritional/M cal-Gavage at-Long-Ev ethylhexy 7737 etric 5:	n, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, -phthalate affect two populations of Leyd Metabolic-Maternal body weights (GD 12 -Duration: Reproductive/Developmental- vans - [rat]-Female I Phthalate- Parent compound <u>Metric</u> Selective Reporting and Attrition	D., Chen, G. ig cells in mal and GD 20 ar 1-F0 - gestation Rating Low	, Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to e Long-Evans rats. Biology of Reproduction 80(5):882-888. nd GD 21.5) on (GD 12.5-GD20)-F0- lactation (PND 0-PND 21) <u>Comments</u> The authors do not report whether or not any animals died over the course of the study
ral-Gavage at-Long-Ev ethylhexy 7737 etric 5:	e-Duration: Reproductive/Developmental- vans - [rat]-Female l Phthalate- Parent compound <u>Metric</u> Selective Reporting and Attrition	1-F0 - gestatio Rating Low	on (GD 12.5-GD20)-F0- lactation (PND 0-PND 21) Comments The authors do not report whether or not any animals died over the course of the study
ethylhexy 7737 etric 5:	vans - [rat]-Female l Phthalate- Parent compound Metric Selective Reporting and Attrition	Rating Low	Comments The authors do not report whether or not any animals died over the course of the study
etric 5:	Metric Selective Reporting and Attrition	Rating Low	Comments The authors do not report whether or not any animals died over the course of the study.
etric 5:	Selective Reporting and Attrition	Low	The authors do not report whether or not any animals died over the course of the study
			and do not report any health outcomes unrelated to the exposure. Not all animals appear to be accounted for in the results, many endpoints report a sample size of N=4-6 or 5-6 per group, despite having 11-13 dams in each group. In Table 2, it seems to be reported that 35-39 pups were in each group, however only 6-13 pups were listed for organ weight data on PND49. These omissions are not explained, and it isn't clear if a large number of pups died during early post-natal development, or if the authors selectively measured endpoints in specific animals. Animals that were not pregnant or did not deliver were excluded. The authors did explain that 1-2 dams from the 10 and 750 mg/kg/day group did not give birth to any pups, but this does not account for the incomplete sampling presented for most outcomes. The significantly smaller sample size reported for most endpoints is a significant concern for data quality. All prespecified outcomes were reported in the results.
ls Sensitiv etric 6:	ity Chemical administration and characterization	Low	The purity of the test substance was not reported and was not analytically determined by the authors. Test substance preparation was described, but storage conditions were omitted. Gavage volume was reported and is 1 mL/kg, which is appropriate. Doses were not analytically confirmed but are reported nominally in mg/kg units. As the purity of the test substance is not reported, these deficiencies are expected to significantly impact the quality of the results.
etric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure is sensitive for endpoints of interest, and covers the window of sensitivity (leydig cell marker detectable beginning GD12.5) for developmental male reproductive health effects.
es and Res	sults Display		
etric 8:	Endpoint sensitivity and specificity	Medium	There are no concerns regarding the test animal species and strains, though there are some concerns regarding the sample size. Animal numbers used (11-13) were fewer than generally recommended by guidance (20) and outcome sample sizes were incom- plete and varied between different endpoints without justification. It is not clear if the authors measured every outcome in every animal due to the inconsistent sample sizes. Outcome assessment methods are described completely and are appropriate. The authors justified their dose-range based off of results seen in previous studies, though the dose range could benefit from using an additional lower dose with the goal of determining a NOAEL.
el	ric 7: s and Re: ric 8:	characterization tric 7: Exposure timing, frequency, and duration s and Results Display tric 8: Endpoint sensitivity and specificity	characterization tric 7: Exposure timing, frequency, and High duration s and Results Display tric 8: Endpoint sensitivity and specificity Medium

PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

	continued from previous page					
Study Citation:	Lin, H., Lia diethylhexy	an, Q., Hu, G., Jin, Y., Zhang, Y., I-phthalate affect two populations o	Hardy, D., Chen, G., f Leydig cells in mal	Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to e Long-Evans rats. Biology of Reproduction 80(5):882-888.		
Health Outcome(s)	Nutritional/	Metabolic-Maternal body weights (GD 12 and GD 20 ar	d GD 21.5)		
Health Effect(s):						
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)					
Species:	Rat-Long-Evans - [rat]-Female					
Chemical:	Diethylhexy	l Phthalate- Parent compound				
Domain	091131	Metric	Rating	Comments		
	Metric 9:	Results presentation	Low	The authors do not specifically report whether or not the litter is the unit of sampling. Reported data (such as in Table 2) suggests that all reported results are from all of the available pups. Most results are reported quantitatively with measures of variance in tables and figures, with some negative data reported qualitatively. Statistical analysis appears to be appropriate and is described in the methods section.		
Additional Comments:	None					
Overall Qualit	ty Deteri	mination	Low			

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888. Reproductive/Developmental-Birth rate in dams and number of pups per dam. Endpoints assessed in pups: male:female ratio, anogenital distance (AGD) at PND2, body weight at PND35 and 49, testes and prostate weight at PND49, Leydig cell histopathology (average, median and maximum number of cells per cluster), testes mRNA expression, protein expression and enzyme activity, serum testosterone levels (PND 21 and 49). Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21) Rat-Long-Evans - [rat]-Female Diethylhexyl Phthalate- Parent compound 697737				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Qu	uality Metric 1:	Reporting Quality	Low	All critical information is reported. Test substance is identified by name, and the sup- plier is reported. The test substance purity was not reported. Test animal species, strain, sex, and commercial source were reported. Starting body weights are reported in the results (table 1). Test animal starting age was not reported but described as "adult" and animal husbandry conditions were not described in any detail, with the authors merely stating that "all animal procedures were performed in accordance with the policies of the Rockefeller University's Animal Care and Use Committee". These deficiencies could significantly impact the quality of the results.	
Domain 2: Selection and	d Performance Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	Low Medium	The authors did not explain how animals were allocated into groups. No measures to reduce observational bias were described, but endpoints of interest are unlikely to be significantly influenced by observational bias as they were not subjective.	
Domain 3: Confounding	g / Variable Cor Metric 4:	ntrol Confounding / Variable Control	Low	An appropriate negative vehicle control is included and there was no response in the control group. Not all information was reported to evaluate confounding (food/water consumption, presence of EDCs in bedding materials) and the authors did not discuss whether measures were taken to reduce exposure to plasticizers. This may have a significant impact on the study results.	
Domain 4: Salaatiya Ba	porting and Att	rition			
	Metric 5:	Selective Reporting and Attrition	Low	The authors do not report whether or not any animals died over the course of the study, and do not report any health outcomes unrelated to the exposure. Not all animals appear to be accounted for in the results, many endpoints report a sample size of N=4-6 or 5-6 per group, despite having 11-13 dams in each group. In Table 2, it seems to be reported that 35-39 pups were in each group, however only 6-13 pups were listed for organ weight data on PND49. These omissions are not explained, and it isn't clear if a large number of pups died during early post-natal development, or if the authors selectively measured endpoints in specific animals. Animals that were not pregnant or did not deliver were excluded. The authors did explain that 1-2 dams from the 10 and 750 mg/kg/day group did not give birth to any pups, but this does not account for the incomplete sampling presented for most outcomes. The significantly smaller sample size reported for most endpoints is a significant concern for data quality. All prespecified outcomes were reported in the results.	
		Conti	nued on ney	xt page	

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697737 Table: 2 of 2

		cont	tinued from p	revious page		
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888. Reproductive/Developmental-Birth rate in dams and number of pups per dam. Endpoints assessed in pups: male:female ratio, anogenital distance (AGD) at PND2, body weight at PND35 and 49, testes and prostate weight at PND49, Leydig cell histopathology (average, median and maximum number of cells per cluster), testes mRNA expression, protein expression and enzyme activity, serum testosterone levels (PND 21 and 49). Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21) Rat-Long-Evans - [rat]-Female Diethylhexyl Phthalate- Parent compound 697737					
Domain		Metric	Rating	Comments		
Domain 5: Exposure M	lethods Sensiti Metric 6:	vity Chemical administration and characterization	Low	The purity of the test substance was not reported and was not analytically determined by the authors. Test substance preparation was described, but storage conditions were omitted. Gavage volume was reported and is 1 mL/kg, which is appropriate. Doses were not analytically confirmed but are reported nominally in mg/kg units. As the purity of the test substance is not reported, these deficiencies are expected to significantly impact		
	Metric 7:	Exposure timing, frequency, and duration	High	the quality of the results. The timing, duration, and frequency of the exposure is sensitive for endpoints of interest, and covers the window of sensitivity (leydig cell marker detectable beginning GD12.5) for developmental male reproductive health effects.		
Domain 6: Outcome M	leasures and Re Metric 8: Metric 9:	esults Display Endpoint sensitivity and specificity Results presentation	Medium	There are no concerns regarding the test animal species and strains, though there are some concerns regarding the sample size. Animal numbers used (11-13) were fewer than generally recommended by guidance (20) and outcome sample sizes were incomplete and varied between different endpoints without justification. It is not clear if the authors measured every outcome in every animal due to the inconsistent sample sizes. Outcome assessment methods are described completely and are appropriate. The authors justified their dose-range based off of results seen in previous studies, though the dose range could benefit from using an additional lower dose with the goal of determining a NOAEL. The authors do not specifically report whether or not the litter is the unit of sampling. Reported data (such as in Table 2) suggests that all reported results are from all of the available pups. Most results are reported qualitatively with measures of variance in tables and figures, with some negative data reported qualitatively. Statistical analysis appears to be appropriate and is described in the methods section.		
Additional Comments:	None					
Overall Quali	ity Deter	mination	Low			

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Study Citation: Health Outcome(s) and Reported Health Effect(s):	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5. Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4). Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Tarminal body weights (Studies 1, 2, 3, and 4). Body weight feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12). Pody weight (Studies 5, 6, 7, and 4).					
	12).					
Duration and Exposure Pouter	Oral-Diet-Du	ration: Reproductive/Developmental-1-F0	- gestation (20 days)		
Species:	Rat-Fischer 3	44 - [rat]-Female				
Chemical:	Diethylhexyl	Phthalate- Parent compound				
HERO ID:	680063	-				
Domain		Metric	Rating	Comments		
Domain 1: Reporting Qu	uality					
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity.		
Domain 2: Selection and	l Performance					
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for mod- ifying factors across groups were noted by the study authors. In addition, for this ex- periment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results.		
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.		

Domain 3: Confounding / Variable Control

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 680063 Table: 1 of 8

		con	tinued from p	revious page			
Study Citation:	Marsman, I B6C3F1 mi	D. S. (1995). NTP technical report on th ce. Toxicity Report Series, vol. 30 30:1-0	e toxicity stud:	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and			
Health Outcome(s) and Reported Health Effect(s):	Reproductiv of live pups gross necroj CoA oxidas nase, bile ac Terminal bo 12).	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and					
Duration and Exposure Route:	Oral-Diet-D	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)					
Species:	Rat-Fischer	344 - [rat]-Female					
Chemical: HERO ID:	Diethylhexy 680063	Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.			
Domain 4: Selective Re	porting and At	trition					
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.			
Domain 5: Exposure M	ethods Sensitiv	vity					
Domani J. Exposure M	Metric 6: Metric 7:	Chemical administration and characterization Exposure timing, frequency, and duration	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results. For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest: however, it is unclear whether the duration of ex-			
		duration		posure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.			
Domain 6: Outcome M	easures and Re	esults Display					

Continued on next page ...

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5. Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage Health Outcome(s) of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, and Reported Health Effect(s): gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4). Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4). Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12). Body weight gain (Studies 5, 6, 7, and 12). **Duration and** Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days) **Exposure Route:** Species: Rat-Fischer 344 - [rat]-Female Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 680063

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an in-utero developmental exposure study, designed as a supplement to larger NTP studies. This study tested limited endpoints, with a focus on evaluating hepatic peroxisome activity. The outcome methods were sensitive to the outcomes of interest for this study; however, there are some concerns with the timing of outcome assessment. The methods state that "during the interval between GDs17-20, maternal livers and pooled fetal livers were weighed and peroxisomal palmitoyl-CoA oxidase activities were measure for 5 rats per group exposed to DEHP and 5 control rats per evaluation day (a total of 15 control rats)." This statement is suggestive of daily sacrifices during that time period; however, each group consisted of only 10 females (so 5/group could not be sacrificed daily). The reported results are derived from 10 dams (equal to the number of animals per group), and the collection time was not included. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values.
	Metric 9:	Results presentation	Medium	Quantitative data (mean \pm SEM) were provided for the endpoints specified for each dose group. Statistical methods were described and were appropriate. The sample sizes are not clearly specified in the data tables for each endpoint. The study text states "For breeding groups in which one female was used for the maximum perinatal exposure determination study, only data for the dam in the in utero exposure study are included." No "exposure determination study" was mentioned for this chemical, it is possible this statement is in error. Relative liver weights were not reported.
Additional Comments:	1. DEHP Su	pp study in utero in rats		
Overall Qualit	y Deteri	nination	Low	

Study Citation:	Marsman, D	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and				
Health Outcome(s) and Reported Health Effect(s): Duration and Evrosure Boute:	B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5. Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge-nase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12). Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)					
Species: Chemical: HERO ID:	Rat-Fischer 344 - [rat]-Female Diethylhexyl Phthalate- Parent compound 680063					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity.		
Domain 2: Selection and	d Performance Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for mod- ifying factors across groups were noted by the study authors. In addition, for this ex- periment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results. Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.		
				encremes et interest nore segeeure et simple incusures.		
Domain 3: Confounding	/ Variable Control Metric 4: Confounding / Variable Control Low The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.					
Continued on next page						

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Human Health Hazard Animal Toxicology Evaluation

		con	tinued from p	revious page		
Study Citation:	Marsman, D B6C3F1 mic	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.				
Health Outcome(s) and Reported Health Effect(s):	Reproductiv of live pups/ gross necrop CoA oxidase nase, bile ac Terminal bo 12).	B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5. Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).				
Duration and Exposure Route:	Oral-Diet-D	uration: Reproductive/Developmental-1-	F0 - gestation (20 days)		
Species: Chemical: HERO ID:	Rat-Fischer 344 - [rat]-Female Diethylhexyl Phthalate- Parent compound 680063					
Domain		Metric	Rating	Comments		
Domain 4: Selective Re	eporting and At Metric 5:	trition Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.		
Domain 5: Exposure M	lethods Sensitiv	vity				
L L	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.		
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.		
Domain 6: Outcome M	easures and Re	sults Display				

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	continued from previous page
Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3E1 mice. Toxicity Report Series, vol. 30 30:1-G5
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12)
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)
Exposure Route:	
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
IIEKO ID:	000003

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an in-utero developmental exposure study, designed as a supplement to larger NTP studies. This study tested limited endpoints, with a focus on evaluating hepatic peroxisome activity. The outcome methods were sensitive to the outcomes of interest for this study; however, there are some concerns with the timing of outcome assessment. The methods state that "during the interval between GDs17-20, maternal livers and pooled fetal livers were weighed and peroxisomal palmitoyl-CoA oxidase activities were measure for 5 rats per group exposed to DEHP and 5 control rats per evaluation day (a total of 15 control rats)." This statement is suggestive of daily sacrifices during that time period; however, each group consisted of only 10 females (so 5/group could not be sacrificed daily). The reported results are derived from 10 dams (equal to the number of animals per group), and the collection time was not included. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values.
	Metric 9:	Results presentation	Medium	Quantitative data (mean \pm SEM) were provided for the endpoints specified for each dose group. Statistical methods were described and were appropriate. The sample sizes are not clearly specified in the data tables for each endpoint. The study text states "For breeding groups in which one female was used for the maximum perinatal exposure determination study, only data for the dam in the in utero exposure study are included." No "exposure determination study" was mentioned for this chemical, it is possible this statement is in error. Relative liver weights were not reported.
Additional Comments:	1. DEHP Su	pp study in utero in rats		
Overall Qualit	y Deteri	nination	Low	

Study Citation:	Marsman, D	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and				
Health Outcome(s) and Reported Health Effect(s): Duration and Evrosure Boute:	B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5. Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge-nase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12). Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)					
Species: Chemical: HERO ID:	Rat-Fischer 344 - [rat]-Female Diethylhexyl Phthalate- Parent compound 680063					
Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity.		
Domain 2: Selection and	d Performance Metric 2: Metric 3:	Allocation Observational Bias / Blinding Changes	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for mod- ifying factors across groups were noted by the study authors. In addition, for this ex- periment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results. Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.		
				encremes et interest nore segeeure et simple incusures.		
Domain 3: Confounding	/ Variable Control Metric 4: Confounding / Variable Control Low The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.					
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		con	tinued from p	revious page		
Study Citation:	Marsman, D B6C3F1 mic	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.				
Health Outcome(s) and Reported Health Effect(s):	Reproductiv of live pups/ gross necrop CoA oxidase nase, bile ac Terminal bo 12).	B6C3F1 mice. Toxicity Report Series, vol. 30/30:1-G5. Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).				
Duration and Exposure Route:	Oral-Diet-D	uration: Reproductive/Developmental-1-	FO - gestation (20 days)		
Species: Chemical: HERO ID:	Rat-Fischer 344 - [rat]-Female Diethylhexyl Phthalate- Parent compound 680063					
Domain		Metric	Rating	Comments		
Domain 4: Selective Re	eporting and At Metric 5:	trition Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.		
Domain 5: Exposure M	ethods Sensitiv	vity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.		
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.		
Domain 6: Outcome M	easures and Re	sults Display				

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.						
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12)						
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)						
Species: Chemical: HERO ID:	Rat-Fischer 344 - [rat]-Female Diethylhexyl Phthalate- Parent compound 680063						

Domain		Metric	Rating	Comments	
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an in-utero developmental exposure study, designed as a supplement to larger NTP studies. This study tested limited endpoints, with a focus on evaluating hepatic peroxisome activity. The outcome methods were sensitive to the outcomes of interest for this study; however, there are some concerns with the timing of outcome assessment. The methods state that "during the interval between GDs17-20, maternal livers and pooled fetal livers were weighed and peroxisomal palmitoyl-CoA oxidase activities were measure for 5 rats per group exposed to DEHP and 5 control rats per evaluation day (a total of 15 control rats)." This statement is suggestive of daily sacrifices during that time period; however, each group consisted of only 10 females (so 5/group could not be sacrificed daily). The reported results are derived from 10 dams (equal to the number of animals per group), and the collection time was not included. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values.	
	Metric 9:	Results presentation	Medium	Quantitative data (mean \pm SEM) were provided for the endpoints specified for each dose group. Statistical methods were described and were appropriate. The sample sizes are not clearly specified in the data tables for each endpoint. The study text states "For breeding groups in which one female was used for the maximum perinatal exposure determination study, only data for the dam in the in utero exposure study are included." No "exposure determination study" was mentioned for this chemical, it is possible this statement is in error. Relative liver weights were not reported.	
Additional Comments: 1. DEHP Supp study in utero in rats					
Overall Quality Determination		nination	Low		

PUBLIC RELEASE DRAFT May 2025 Human Health Hazard Animal Toxicology Evaluation

Study Citation:	Marsman, D	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and						
Health Outcome(s) and Reported Health Effect(s):	B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5. Other (please specify below) (Clinical observations)-Clinical Observations							
Duration and	Oral-Diet-Du	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)						
Species: Chemical: HERO ID:	Rat-Fischer (Diethylhexyl 680063							
Domain		Metric	Rating	Comments				
Domain 1: Reporting Q	uality							
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity.				
Domain 2: Selection an	d Performance							
Domain 2. Selection an	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for mod- ifying factors across groups were noted by the study authors. In addition, for this ex- periment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results.				
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.				
Demain 21 Confounding (Verichle Control								
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.				
Domain 4: Selective Reporting and Attrition								
		Со	ntinued on next page					
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		c	continued from previous	page
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Marsman, E B6C3F1 mic Other (pleas	D. S. (1995). NTP technical report on the to ce. Toxicity Report Series, vol. 30 30:1-G5. e specify below) (Clinical observations)-Cli	oxicity studies of dibutyl	phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Duration and Exposure Boute:	Oral-Diet-D	uration: Reproductive/Developmental-1-F0	- gestation (20 days)	
Species: Chemical: HERO ID:	Rat-Fischer Diethylhexy 680063	344 - [rat]-Female l Phthalate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.
Domain 5: Exposure M	ethods Sensitiv	rity		
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.
Domain 6: Outcome M	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an in utero developmental exposure study and dams were observed for clinical signs. The frequency of observations was reported, and it was specified that results were recorded "as needed." The test model, including the source and strain were appropriate for the evaluation of the endpoints. The sample size (10 pregnant females/group) was small, but sufficient for performing statistics.
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for clinical signs.
Additional Comments:	1. DEHP Su	pp study in utero in rats		
Overall Ouali	tv Deterr	nination	Uninformative	

Study Citation:	Marsman, E	D. S. (1995). NTP technical report on the t	oxicity stud	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	B6C3F1 mid Reproductiv of live pups/ gross necrop CoA oxidaso nase, bile ac Terminal bo 12). Oral-Diet-D Rat-Fischer Diethylhexy 680063	ce. Toxicity Report Series, vol. 30 30:1-G5. e/Developmental-No. fetuses/breeding gro /litter, number of pups/sex/litter, Offspring co osy, offspring body weights, number of impla e activity of dams (Studies 1, 2, 3, and 4).Ser ids, and glucose), Histopathology of liver (S dy weights (Studies 1, 2, 3, and 4).Body we uration: Reproductive/Developmental-1-F0- 344 - [rat]-Female 1 Phthalate- Parent compound	up, Litter w clinical obse antation sites um chemistr tudies 8 and ight, feed co - lactation (2	eight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 21 days)
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Re	porting and At	trition		
		Conti	nued on nex	at page

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Study Citation:	Marsman, I	D. S. (1995). NTP technical report on the	toxicity stud	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Health Outcome(s) and Reported Health Effect(s):	Reproductiv of live pups gross necrop CoA oxidas nase, bile ac Terminal bo 12).	ce. Toxicity Report Series, vol. 30 30:1-0. ye/Developmental-No. fetuses/breeding gr /litter, number of pups/sex/litter, Offspring psy, offspring body weights, number of imp e activity of dams (Studies 1, 2, 3, and 4).Sec rids, and glucose), Histopathology of liver (bdy weights (Studies 1, 2, 3, and 4).Body w	o. roup, Litter w g clinical obse lantation sites erum chemistr Studies 8 and reight, feed co	reight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, , mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and
Duration and	Oral-Diet-D	Ouration: Reproductive/Developmental-1-F	0-lactation (2	21 days)
Species:	Rat-Fischer	344 - [rat]-Female		
Chemical:	Diethylhexy	/l Phthalate- Parent compound		
HERO ID:	680063			
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.
Domain 5: Exposure M	ethods Sensitiv	vity		
Domain of Exposure in	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The window of exposure (days 1-22 of lactation) differs from standard developmental studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome M	easures and Re	esulte Dienlav		
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.
		Cont	tinued on nex	xt page

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Human Health Hazard Animal Toxicology Evaluation

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Study Citation: Health Outcome(s) and Reported Health Effect(s):	Marsman, D B6C3F1 mic Reproductive of live pups/ gross pecrop	. S. (1995). NTP technical report on the e. Toxicity Report Series, vol. 30 30:1-G e/Developmental-No. fetuses/breeding gr litter, number of pups/sex/litter, Offspring sy offspring body weights number of imp	toxicity stud 5. oup, Litter w clinical obse	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and eight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, mating index_fertility index_Hepatic/Liver_Absolute liver weights of dams_palmitovl-
	CoA oxidase nase, bile aci Terminal boo 12).	activity of dams (Studies 1, 2, 3, and 4).See ds, and glucose), Histopathology of liver (ly weights (Studies 1, 2, 3, and 4).Body w	erum chemistr Studies 8 and eight, feed co	y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and
Duration and	Oral-Diet-Di	aration: Reproductive/Developmental-1-F	0- lactation (2	21 days)
Exposure Route: Species:	Rat-Fischer	344 - [rat]-Female		
Chemical:	Diethylhexy	Phthalate- Parent compound		
HERO ID:	680063			
Domain		Metric	Rating	Comments
	Metric 9:	Results presentation	Medium	Quantitative data (mean \pm SEM) were provided for the endpoints Day 0 litter weight, individual pup body weight (Day 7, 14, 21), pup absolute liver weight, palmitoyl-CoA oxidase activity in livers of pups, absolute liver weights of dams, palmitoyl-CoA ox- idase activity in dam livers, and terminal body weights of dams. Statistical signifi- cance was provided for these endpoints. In addition, the study groups were clearly provided/indicated. It should be noted that the day of parturition and day of lactation varied between the exposure groups. As a result, two sets of analyses were performed for each day of evaluation. Data for the following endpoints: sex and number of pups and litter weights on LD0 and 1; number, sex, and individual pup body weights on LD4, were not provided.
Additional Comments:	2.DEHP Sup	p study lactational in rats		
Overall Qualit	v Detern	nination	Low	

Study Citation:	Marsman, I	D. S. (1995). NTP technical report on the t	oxicity stud	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	B6C3F1 min Reproductiv of live pups, gross necrop CoA oxidase nase, bile ac Terminal bo 12). Oral-Diet-D Rat-Fischer Diethylhexy 680063	ce. Toxicity Report Series, vol. 30 30:1-G5. re/Developmental-No. fetuses/breeding gro /litter, number of pups/sex/litter, Offspring of osy, offspring body weights, number of impla e activity of dams (Studies 1, 2, 3, and 4).Ser ids, and glucose), Histopathology of liver (S dy weights (Studies 1, 2, 3, and 4).Body we puration: Reproductive/Developmental-1-F0- 344 - [rat]-Female 1 Phthalate- Parent compound	up, Litter w clinical obse intation sites um chemistr tudies 8 and ight, feed co - lactation (2	eight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, , mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 21 days)
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Re	porting and At	trition		
		Contin	nued on nex	at page

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Human Health Hazard Animal Toxicology Evaluation

		cont	inued from p	revious page
Study Citation:	Marsman, I B6C3E1 mi	D. S. (1995). NTP technical report on the	toxicity stud	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Health Outcome(s) and Reported Health Effect(s):	Reproductiv of live pups, gross necrop CoA oxidase nase, bile ac Terminal bo 12).	ve/Developmental-No. fetuses/breeding gr /litter, number of pups/sex/litter, Offspring posy, offspring body weights, number of imp e activity of dams (Studies 1, 2, 3, and 4).Se ids, and glucose), Histopathology of liver (dy weights (Studies 1, 2, 3, and 4).Body w	youp, Litter w golinical obse lantation sites erum chemistr Studies 8 and reight, feed co	reight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, , mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- onsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and
Duration and Exposure Route:	Oral-Diet-D	puration: Reproductive/Developmental-1-F	0- lactation (2	21 days)
Species:	Rat-Fischer	344 - [rat]-Female		
Chemical:	Diethylhexy	Phthalate- Parent compound		
HERO ID:	680063			
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.
Domain 5: Exposure M	ethods Sensitiv	vity		
	Metric 7:	Chemical administration and characterization	Low High	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results. The window of exposure (days 1-22 of lactation) differs from standard developmental
	Weule 7.	duration	Ingn	studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome M	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.
		Cont	tinued on nex	xt page

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 680063 Table: 6 of 8

		C	ontinued from p	revious page
Study Citation:	Marsman, D	. S. (1995). NTP technical report on Toxicity Perport Series, vol. 30 30:1	the toxicity stud	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Health Outcome(s) and Reported Health Effect(s):	Reproductiv of live pups/ gross necrop CoA oxidase nase, bile ac Terminal boo 12).	e/Developmental-No. fetuses/breeding litter, number of pups/sex/litter, Offspi sy, offspring body weights, number of i e activity of dams (Studies 1, 2, 3, and 4 ids, and glucose), Histopathology of liv dy weights (Studies 1, 2, 3, and 4).Bod	g group, Litter w ring clinical obse mplantation sites).Serum chemistr er (Studies 8 and y weight, feed co	eight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, , mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and
Duration and	Oral-Diet-D	uration: Reproductive/Developmental-	1-F0- lactation (2	21 days)
Exposure Route:				
Species:	Rat-Fischer	344 - [rat]-Female		
HERO ID:	680063	i Philaiate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 9:	Results presentation	Medium	Quantitative data (mean \pm SEM) were provided for the endpoints Day 0 litter weight, individual pup body weight (Day 7, 14, 21), pup absolute liver weight, palmitoyl-CoA oxidase activity in livers of pups, absolute liver weights of dams, palmitoyl-CoA ox- idase activity in dam livers, and terminal body weights of dams. Statistical signifi- cance was provided for these endpoints. In addition, the study groups were clearly provided/indicated. It should be noted that the day of parturition and day of lactation varied between the exposure groups. As a result, two sets of analyses were performed for each day of evaluation. Data for the following endpoints: sex and number of pups and litter weights on LD0 and 1; number, sex, and individual pup body weights on LD4, were not provided.
Additional Comments:	2.DEHP Sup	op study lactational in rats		
Overall Quali	ty Deterr	nination	Low	

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Study Citation:	Marsman, D	D. S. (1995). NTP technical report on the t	oxicity stud	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	B6C3F1 mid Reproductiv of live pups/ gross necrop CoA oxidaso nase, bile ac Terminal bo 12). Oral-Diet-D Rat-Fischer Diethylhexy 680063	ce. Toxicity Report Series, vol. 30 30:1-G5. e/Developmental-No. fetuses/breeding gro /litter, number of pups/sex/litter, Offspring co osy, offspring body weights, number of impla e activity of dams (Studies 1, 2, 3, and 4).Ser ids, and glucose), Histopathology of liver (S dy weights (Studies 1, 2, 3, and 4).Body we uration: Reproductive/Developmental-1-F0- 344 - [rat]-Female 1 Phthalate- Parent compound	up, Litter w clinical obse antation sites um chemistr tudies 8 and ight, feed co - lactation (2	eight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 21 days)
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and	d Performance Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding	g / Variable Co Metric 4:	ntrol Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Re	porting and At	trition		
		Contin	nued on nex	at page

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 680063 Table: 7 of 8

		cont	inued from p	revious page
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Marsman, I B6C3F1 mi Reproductiv of live pups gross necrop CoA oxidas nase, bile ac Terminal bo 12).	D. S. (1995). NTP technical report on the ce. Toxicity Report Series, vol. 30 30:1-G. ve/Developmental-No. fetuses/breeding gr/litter, number of pups/sex/litter, Offspring body weights, number of imp e activity of dams (Studies 1, 2, 3, and 4).So ids, and glucose), Histopathology of liver (dy weights (Studies 1, 2, 3, and 4).Body w	e toxicity stud 5. roup, Litter w g clinical obse olantation sites erum chemistr (Studies 8 and veight, feed co	ies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and reight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, a mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and
Duration and	Oral-Diet-D	ouration: Reproductive/Developmental-1-F	FO- lactation (2	21 days)
Exposure Route:	Rat-Fischer	344 - [rat]-Female		
Chemical: HERO ID:	Diethylhexy 680063	Phthalate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.
Domain 5: Exposure N	lethods Sensitiv	vity		
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The window of exposure (days 1-22 of lactation) differs from standard developmental studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome M	acures and De	sults Display		
Domain of Outcome iv	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.
		Con	tinued on nex	at page

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Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

	c	ontinued from p	revious page
Study Citation: Health Outcome(s) and Reported Health Effect(s):	Marsman, D. S. (1995). NTP technical report on B6C3F1 mice. Toxicity Report Series, vol. 30 30:1 Reproductive/Developmental-No. fetuses/breeding of live pups/litter, number of pups/sex/litter, Offsp gross necropsy, offspring body weights, number of i CoA oxidase activity of dams (Studies 1, 2, 3, and 4 nase, bile acids, and glucose), Histopathology of liv Terminal body weights (Studies 1, 2, 3, and 4).Bod 12).	the toxicity studi -G5. g group, Litter w ring clinical obser implantation sites).Serum chemistr er (Studies 8 and y weight, feed co	eight; Gestation length, number of pups/litter, number of live pups/litter, percentage rvations, mortality, feed consumption, histologic examinations on >30 organs/tissues, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl- y (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydroge- 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11)Nutritional/Metabolic- nsumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and
Duration and	Oral-Diet-Duration: Reproductive/Developmental-	1-F0- lactation (2	1 days)
Exposure Route:			
Species:	Rat-Fischer 344 - [rat]-Female		
HERO ID.	680063		
		D ('	
Domain	Metric Descrite and station	Rating	Comments
	Metric 9: Results presentation	Medium	Quantitative data (mean \pm SEM) were provided for the endpoints Day 0 litter weight, individual pup body weight (Day 7, 14, 21), pup absolute liver weight, palmitoyl-CoA oxidase activity in livers of pups, absolute liver weights of dams, palmitoyl-CoA ox- idase activity in dam livers, and terminal body weights of dams. Statistical signifi- cance was provided for these endpoints. In addition, the study groups were clearly provided/indicated. It should be noted that the day of parturition and day of lactation varied between the exposure groups. As a result, two sets of analyses were performed for each day of evaluation. Data for the following endpoints: sex and number of pups and litter weights on LD0 and 1; number, sex, and individual pup body weights on LD4, were not provided.
Additional Comments:	2.DEHP Supp study lactational in rats		
Overall Qualit	y Determination	Low	

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Study Citation: Health Outcome(s) and Reported	Marsman, D B6C3F1 mic Other (please	 S. (1995). NTP technical report on the toxic re. Toxicity Report Series, vol. 30 30:1-G5. e specify below) (Clinical observations)-Clinical 	ity studies of dibuty Il Observations	el phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and					
Health Effect(s): Duration and	Oral-Diet-D	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)							
Exposure Route: Species:	Rat-Fischer	344 - [rat]-Female							
Chemical:	Diethylhexy	l Phthalate- Parent compound							
HERO ID:	680063	ľ							
Domain		Metric	Rating	Comments					
Domain 1: Reporting Q	Quality								
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study in- cluded identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantita- tive and qualitative). The study lacked the starting body weights of the dams and did no report parity. The missing information is not expected to significantly impact the study evaluation.					
Domain 2: Selection ar	d Darformanca								
Domain 2. Selection ai	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors.					
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.					
Domain 2: Confoundin	a / Variabla Cos	atral							
Boniani 5. Comoundin	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a know endocrine disruptor.					
Domain 4. Salasting D	monting and Au	uition							
Domain 4: Selective K	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litt weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/grou however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition					

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 680063 Table: 8 of 8

Study Citation: Marsman B6C3F1 Health Outcome(s) Other (pl and Reported Health Effect(s): Duration and Duration and Oral-Diet Exposure Route: Species: Species: Rat-Fisch Chemical: Diethylho HERO ID: 680063 Domain Domain	, D. S. (1995). NTP technical report on the to mice. Toxicity Report Series, vol. 30 30:1-G5. ease specify below) (Clinical observations)-Clin -Duration: Reproductive/Developmental-1-F0-	oxicity studies of dibutyl	phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and			
Health Outcome(s) Other (pl and Reported Health Effect(s): Duration and Oral-Diet Exposure Route: Species: Rat-Fisch Chemical: Diethylhe HERO ID: 680063 Domain Domain 5: Exposure Methods Sens Metric 6:	ease specify below) (Clinical observations)-Clinical-Duration: Reproductive/Developmental-1-F0-	nical Observations				
Duration and Oral-Diet Exposure Route: Species: Species: Rat-Fisch Chemical: Diethylho HERO ID: 680063 Domain Domain Domain 5: Exposure Methods Sens Metric 6: Metric 6:	-Duration: Reproductive/Developmental-1-F0-					
Species: Rat-Fisch Chemical: Diethylho HERO ID: 680063 Domain Domain 5: Exposure Methods Sens Metric 6:		lactation (21 days)				
Chemical: Diethylho HERO ID: 680063 Domain	Rat-Fischer 344 - [rat]-Female					
Domain Domain 5: Exposure Methods Sens Metric 6:	exyl Phthalate- Parent compound					
Domain 5: Exposure Methods Sens Metric 6:	Metric	Rating	Comments			
Metric 6:	itivity					
Metric 7:	Chemical administration and characterization Exposure timing, frequency, and	Low High	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results. The window of exposure (days 1-22 of lactation) differs from standard developmental			
	duration	C	studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.			
Domain 6: Outcome Measures and	Results Display					
Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.			
Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for clinical signs.			
Additional Comments: 2.DEHP	Supp study lactational in rats					
Avorall Auglity Doto		I Ininformation				

Study Citation: Health Outcome(s) and Reported	Martino-And of active pht Reproductive	Martino-Andrade, A. J., Morais, R. N., Botelho, G. G., Muller, G., Grande, S. W., Carpentieri, G. B., Leao, G. M., Dalsenter, P. R. (2008). Coadministration of active phthalates results in disruption of foetal testicular function in rats. International Journal of Andrology 32(6):704-12. Reproductive/Developmental-Testicular testosterone levels					
Health Effect(s): Duration and Exposure Route:	Oral-Gavage	e-Duration: Reproductive/Developmental-F0	- gestation (GD	013-GD21)			
Species: Chemical: HERO ID:	Rat-Wistar - Diethylhexy	[rat]-Female l Phthalate- Parent compound					
Domain	070201	Metric	Rating	Comments			
Domain 1: Reporting Qu	uality Metric 1:	Reporting Quality	Medium	All critical and most important information was reported. Reported information in- cluded information on the test substance (name, CASRN, purity and source), the test model (species, strain, sex, and source), animal husbandry details (photoperiod, tem- perature, food and water availability), exposure methods, experimental design, endpoint evaluations, and presentation of results.Missing information included test animal age, initial body weights, parity, humidity, and number of animals per cage.			
Domain 2: Selection and	l Performance Metric 2:	Allocation	Low	No details on the allocation of dams into study groups or on the selection of fetuses for outcome analysis were provided.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcome was measured using a standard laboratory kit.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included and gave the expected response. There were no differences in maternal body weights (fetal weights were not measured) and gavage volumes were consistent across groups. No differences in the animal husbandry parameters reported were noted. It is unclear whether the study took measures to minimize the exposure to other plasticizers (e.g., from cage, bedding, or water dispensing materials, or in food), which could influence the study results.			
Domain 4: Selective Rep	porting and Att Metric 5:	trition Selective Reporting and Attrition	Medium	The number of dams used in the study was reported as a range (6-9 per group). It was not specified if any of the dams used for this endpoint died. Data were reported for the endpoint of interest and the sample sizes for the from 6-8 (litters)/group. Based on the information provided, there is no evidence of selective reporting.			
Domain 5: Exposure Me	ethods Sensitiv	ity					
	Continued on next page						

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 676281 Table: 1 of 1

			nava nom prom	Puese		
Study Citation:	Martino-Andrade, A. J., Morais, R. N., Botelho, G. G., Muller, G., Grande, S. W., Carpentieri, G. B., Leao, G. M., Dalsenter, P. R. (2008). Coadministration of active phthalates results in disruption of foetal testicular function in rats. International Journal of Andrology 32(6):704-12.					
Health Outcome(s)	Reproductiv	ve/Developmental-Testicular testosterone lev	vels			
and Reported						
Health Effect(s):						
Duration and	Oral-Gavage	e-Duration: Reproductive/Developmental-F	0 - gestation (GD	013-GD21)		
Exposure Route:						
Species:	Rat-Wistar -	[rat]-Female				
Chemical:	Diethylhexy	l Phthalate- Parent compound				
HERO ID:	676281					
Domain		Metric	Rating	Comments		
	Metric 6:	Chemical administration and characterization	Medium	The test material source (Sigma-Aldrich) and purity (99%) were reported. The study did not include the certificate of analysis (or catalogue number), and the test material was not verified by the performing laboratory. Certificates of analysis are generally available on the supplier's website. Animals were dosed via gavage in corn oil and the gavage volume (5mL/kg) was appropriate. No details on the preparation, storage, or stability of the test solutions were provided. Doses were reported in mg/kg-day. It is not specified whether doses were adjusted daily based on measured body weights. Doses were not analytically verified.		
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD13-21. This exposure covers the period of post- implantation embryonic development, and the critical windows of organogenesis and male sexual differentiation.		
Domain 6: Outcome M	easures and Re	sults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Testosterone levels were measured using an ELISA assay from presumably pooled sam- ples from the right testes of 1-2 males (GD21) per litter, and 6-8 litters per dose. There are no major concerns about the sample size used. The study text noted that samples were measured in a single run, suggesting the lack of replicates. Only a single dose was tested. The dose selected was either not expected to suppress testicular testosterone lev- els, or only to produce small changes and was justified by the authors. The study was focused on other endpoints that were presumably more sensitive. There are no concerns about the test model used.		
	Metric 9:	Results presentation	High	Results were reported in a figure (bar graph) showing means \pm SEM. Statistical significance and sample size (number of litters and individual fetuses) were shown. Litters were used as the experimental unit. Individual animal data were not provided		
Additional Comments:	None					

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Rajagopal, G., Bhaskaran, R. S., Karundevi, B. (2019). Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat F1 male offspring. Journal of Applied Toxicology 39(5):751-763. Reproductive/Developmental-F1 males: blood glucose, serum insulin, insulin resistance, body weight, serum AST, ALT, ALP; hepatic glycogen concentration; enzymatic activity (i.e., glycogen synthase; glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); protein expression (i.e., Beta-arrestin; c-Src; phosphorylated/non-phosphorylated IR-beta, IRS-1, AKT, FoxO1, GSK3beta); mRNA levels (i.e., glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); transcription factor FoxO1 interaction with gene promoters glucose-6-phosphatase and phosphoenolpyruvate carboxykinase; serum urea, creatinine, testosterone and estradiol. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)-F0- lactation (PND 1-21) Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 5507636 						
Domain 1: Domartic = O	molity	Metric	Kating	Comments			
	Metric 1:	Reporting Quality	Medium	All critical and some important information were reported. The test material was identified as di-(2-ethylhexyl) phthalate (DEHP). The vendor source was reported; the purity was not specifically stated, but since the substance was analytical grade, a purity of \geq 99.50 was obtained from the product specifications reported by the vendor. Test model (species, strain, parity, and age of the dams) and animal husbandry (cage type, light/dark cycle schedule, and food and water availability) details were reported. The experimental design including the number of animals per group, endpoint evaluation methods, and quantitative results for all outcomes were reported. No specific testing guideline or indication of GLP was reported. Missing animal husbandry information included temperature and humidity, and animal source was not reported.			
Domain 2: Salastion on	d Darformanaa						
Domain 2. Selection an	Metric 2:	Allocation	Low	No information on the methods of allocation or randomization of animals into test groups, or for the selection of males used to assess specific endpoints was provided.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature.			
Domain 3: Confoundin	Domain 3: Confounding / Variable Control						
	Meuric 4:	Contounding / variable Control	Low	appropriate. No positive control was used. No body weight or food consumption data were reported for the dams. General animal husbandry conditions and procedures that were reported were limited to cage type, light/dark cycle schedule, and food and water availability, and these were consistent across groups. It was not reported whether mea- sures were taken to minimize exposure to other plasticizers in food, bedding, or water dispensing and caging materials. The test substance is a known endocrine disrupter.			
Domain 4: Selective Re	porting and At	trition					
Continued on next page							

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 5507636 Table: 1 of 1

		conti	inued from previ	ious page		
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Rajagopal, G., Bhaskaran, R. S., Karundevi, B. (2019). Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat F1 male offspring. Journal of Applied Toxicology 39(5):751-763. Reproductive/Developmental-F1 males: blood glucose, serum insulin, insulin resistance, body weight, serum AST, ALT, ALP; hepatic glycogen concentration; enzymatic activity (i.e., glycogen synthase; glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); protein expression (i.e., Beta-arrestin; c-Src; phosphorylated/non-phosphorylated IR-beta, IRS-1, AKT, FoxO1, GSK3beta); mRNA levels (i.e., glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); transcription factor FoxO1 interaction with gene promoters glucose-6-phosphatase and phosphoenolpyruvate carboxykinase; serum urea, creatinine, testosterone and estradiol. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)-F0- lactation (PND 1-21) Rat-Wistar - [rat]-Female Diethylhexyl Phthalate- Parent compound 5507636					
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	Medium	Results were reported for all outcomes and sample sizes were clearly described and included in the results. Insufficient information was provided to determine animal attri- tion. No mortality results or details of animal health were provided. Since the sample sizes were small (6 out of 36 males/group were used for most endpoints), it cannot be determined whether any animals died.		
Damain 5. Emanuel M	-4h - J- C:4:-					
Domain 5: Exposure M	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP via oral gavage. The vendor source was reported; the purity was not specifically stated, but since the substance was analytical grade, a purity of \geq 99.50 was obtained from the product specifications reported by the vendor. The lot number, preparation details, and storage conditions of the test solutions were not reported. The route and method of exposure were suited to the test substance. Doses in mg/kg-day were provided; there is no indication that the test solutions were analytically verified. The gavage volume was not reported.		
	Metric 7:	Exposure timing, frequency, and duration	High	The gavage dosing frequency (daily) and duration are considered adequate. The expo- sure timing of the dams from gestational day 9 to postnatal day 21 (lactation period) does not adhere to a specific reproductive/developmental testing guideline (e.g., OECD 414, 421), but this study was specifically interested in studying the effects of gestational and lactational exposure on insulin signalling and glucoregulatory effects.		
Joinani o: Outcome M	Metric 8:	Endpoint sensitivity and specificity	Medium	Only two exposure groups were tested but spacing was adequate to observe a concentration-related response. However, the authors did not justify the selected doses and the spacing was not adequate to identify a NOAEL (effects observed in all treatment groups). The source of the animals was not reported. Only 6 dams per group were tested, and only male F1 offspring were evaluated without justification. For most endpoints, only 1 male per dam was tested (n = 6/group); therefore, the study was not designed to assess litter effects. The sample sizes were sufficient to conduct statistical analysis. No testing guideline was followed. The study had a narrow focus and therefore did not include an assessment of any maternal endpoints including food intake, or, clinical signs, organ weights, macroscopic evaluations or histology of the target organs/systems in either dams or offspring. F1 males were appropriately fasted prior to nutritional/metabolic assessments (i.e., blood glucose, serum insulin, insulin resistance). The methods of the outcome assessment were clearly described and were consistent across groups.		
		Cont	inued on next pa	age		

Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 5507636 Table: 1 of 1

	•••	continued from previ	bus page			
Study Citation:	Rajagopal, G., Bhaskaran, R. S., Karundevi, B. glucoregulatory events in rat F1 male offspring. Jo	Rajagopal, G., Bhaskaran, R. S., Karundevi, B. (2019). Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat El male offspring. Journal of Applied Toxicology 39(5):751,763				
Health Outcome(s)	Reproductive/Developmental-F1 males: blood glu	cose, serum insulin, in	sulin resistance, body weight, serum AST, ALT, ALP; hepatic glycogen concen-			
and Reported	tration; enzymatic activity (i.e., glycogen synthas	e; glucose-6-phosphata	ase; phosphoenolpyruvate carboxykinase); protein expression (i.e., Beta-arrestin;			
Health Effect(s):	c-Src; phosphorylated/non-phosphorylated IR-bet carboxykinase); transcription factor FoxO1 interact creatining, testosterone and setradial	a, IRS-1, AKT, FoxOl ction with gene promot	I, GSK3beta); mRNA levels (i.e., glucose-6-phosphatase; phosphoenolpyruvate ters glucose-6-phosphatase and phosphoenolpyruvate carboxykinase; serum urea,			
Duration and	Oral-Gavage-Duration: Reproductive/Developmer	ntal-1-F0 - gestation (G	D 9-21)-F0- lactation (PND 1-21)			
Exposure Route:		2				
Species:	Rat-Wistar - [rat]-Female					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	5507636					
Domain	Metric	Rating	Comments			
	Metric 9: Results presentation	Medium	Results were adequately reported in the text and quantitatively. Data were presented as means \pm standard error and sample sizes were included in each figure. Western blot figures of the protein bands were not labeled to indicate the specific dose group. Statistical significance is shown and statistical methods were described and appropriate. Individual animal data were not provided. It would have been appropriate to assess some endpoints (e.g., birth weights) using the litter as the statistical unit; however, litters were culled, presumably immediately, to 6 males per litter, and only 1 male per litter was used for any given endpoint.			
Additional Comments:	None					
Overall Quali	ty Determination	Medium				

Study Citation:	Rajesh, P., Balasubramanian, K. (2014). Phthal 223(1):47-66.	late exposure in utero causes epigen	etic changes and impairs insulin signalling. Journal of Endocrinology
Health Outcome(s)	Nutritional/Metabolic-Apical endpoints: Lean b	oody weight, fat weight, fasting bloc	d glucose and insulin levels. Mechanistic endpoints: gene expression,
and Reported	epigenetic modification (DNA methylation, ChI	P), protein levels (Western, immuno	histochemistry) of molecules involved in insulin signalling and glucose
Health Effect(s):	regulation.		
Duration and	Oral-Gavage-Duration: Reproductive/Developm	nental-1-F0 - gestation (GD 9-21)	
Exposure Route:			
Species:	Rat-Wistar - [rat]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	2519077		
Domain	Metric	Rating	Comments
Demain 1. Demanting O			

		0	
Domain 1: Reporting Quality			
	1: Reporting Quality	Medium	All critical information was reported. The test material was Di-(2-ethylhexyl)phthalate (DEHP), doses (1, 10, and 100 mg/kg-day), duration (GD 9-21), route (oral, gavage), and test species (Wistar rats) were reported, and quantitative results were reported for all outcomes. Important information included the test animal age, parity, sex, starting body weights, number of animals per group ($n = 6$), and detailed endpoint evaluation methods. The test substance source was not clearly specified; the study indicated that the chemicals used in the study were purchased from "Sigma chemical company, Amersham Biosciences, and Sisco Research Laboratories." It is unclear which source DEHP was from. The purity of all reagents was of molecular and analytical grade. The animal source was not specified. Limited animal husbandry conditions were specified; it was only noted that pregnant females were placed in individual cages and given access to food and water ad libitum. However, the text did indicate that animals were maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethical Committee (IAEC no. 01/01/2010). The missing information is not expected to have a major impact on the study results.
Domain 2: Selection and Perform	ance		
Metric	2: Allocation	Low	The method of allocation of animals into groups was not specified. It was also not indi- cated if the offspring used for certain outcomes (e.g., oral glucose and insulin tolerance tests) were randomly selected.
Metric	3: Observational Bias / Blinding Changes	Medium	Methods to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes were not subjective in nature or were simple measures (lean body weight, fat weight etc.).
Domain 3: Confounding / Variabl	e Control		
Metric ·	4: Confounding / Variable Control	Medium	The study did not report some of the standard measures that could bias results (e.g., food and water intake, or details of animal husbandry). Differences in lean body weights were observed, but the changes are presumed to be related to exposure. The chemical of interest is an endocrine disruptor, and it was not specified whether measures were taken to minimize exposure to other plasticizers. Concurrent negative vehicle (olive oil) controls were used and the biological responses were appropriate. Positive controls were not necessary because many of the effects of DEHP were known, and this study was focused more on the mechanisms of action.

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Diethylhexyl Phthalate

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Study Citation:	Rajesh, P., B	alasubramanian, K. (2014). Phthalate exp	osure in utero cau	ses epigenetic changes and impairs insulin signalling. Journal of Endocrinology		
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species:	223(1):47-66. Nutritional/Metabolic-Apical endpoints: Lean body weight, fat weight, fasting blood glucose and insulin levels. Mechanistic endpoints: gene expression, epigenetic modification (DNA methylation, ChIP), protein levels (Western, immunohistochemistry) of molecules involved in insulin signalling and glucose regulation. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)					
Chemical: HERO ID:	Diethylhexyl 2519077	Phthalate- Parent compound				
Domain		Metric	Rating	Comments		
Domain 4: Selective Rep	porting and Att Metric 5:	rition Selective Reporting and Attrition	Medium	Mortality, pup survival, or survival of offspring until the end of the study were not mea- sured endpoints. For one outcome (glucose and insulin tolerance tests), it specifies that the tests were conducted on 6 male and 6 female offspring from different litters with only 1 offspring/sex/litter); since there were 6 pregnant females exposed per group, this suggests that at least all of the treated females survived and that six litters were gener- ated. Results for all pre-specified outcomes were reported, and there was no indication of selective reporting.		
Domain 5: Exposure Me	ethods Sensitivi	ity				
Domain of Exposure inc	Metric 6:	Chemical administration and characterization	Medium	The exact chemical source cannot be determined; however, all of the listed sources are appropriate. There was no independent analytical verification of the test substance or the purity, and the doses were not analytically verified. No information on the preparation of the test solutions, confirmation of homogeneity, or stability was provided. The exposure route (gavage) was appropriate for the test substance. The gavage volume was 2.0 mL/kg bw and the dosage was adjusted daily for maternal body weight changes. The noted uncertainties are expected to have a minimal impact on the interpretation of the results.		
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration (daily from GD 9-21 or parturition) were reported and were appropriate for the purposes of the study, although beyond indicating the desire to expose during gestation, the authors did not justify the selected window. All animals were dosed at the same time of day.		
Domain 6: Outcome Me	essures and Res	ulte Dieplay				
Johan o. Outcome Me	Metric 9:	Endpoint sensitivity and specificity Results presentation	Medium High	The study used Wistar rats. The use of the specific strain was not justified, but the ani- mals appeared to be appropriate. This was not a guideline study, so there were no rules for the number of animals used. The study used 6 pregnant females/dose and culled each litter to 4/sex on PND1. All of the protocols were described in sufficient detail and were sensitive to the outcomes of interest. The study used three dose groups, plus a control, allowing for the data to show a dose response. The doses/spacing did not allow for the determination of a NOAEL (significant effects were observed in all dose groups), but this was not a goal of the study. The sample sizes were sufficient to allow for statistical analysis, and animals from each litter were represented. The outcomes were consistently assessed across groups. Data were reported by sex for all dose groups, as means \pm SE; the sample size (n) and		
				statistical significance were noted. Statistical methods were clearly described and appro- priate for the datasets.		

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Human Health Hazard Animal Toxicology Evaluation

		continued from previous page				
Study Citation:	Rajesh, P., Balasubramanian, K. (2014). Phthalate exposure in utero causes epigenetic changes and impairs insulin signalling. Journal of Endocrinology 223(1):47-66					
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Apical endpoints: Lean body weight, fat weight, fasting blood glucose and insulin levels. Mechanistic endpoints: gene expression, epigenetic modification (DNA methylation, ChIP), protein levels (Western, immunohistochemistry) of molecules involved in insulin signalling and glucose regulation.					
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)					
Species:	Rat-Wistar - [rat]-Female					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	2519077					
Domain	Metric	Rating	Comments			
Additional Comments:	None					
Overall Qualit	ty Determination	Medium				

Study Citation:	Saillenfait, A	. M., Sabaté, J. P., Robert, A., Rouiller-Fab	ore, V., Roud	ot, A. C., Moison, D., Denis, F. (2013). Dose-dependent alterations in gene expression			
Health Outcome(s) and Reported Health Effect(s):	Reproductive	and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. Journal of Applied Toxicology 33(9):1027-1035. Reproductive/Developmental-Fetal ex vivo testosterone production.					
Duration and	Oral-Gavage	-Duration: Reproductive/Developmental-1-	-F0 - gestatio	on (GD12-19)			
Exposure Route:	C		C				
Species:	Rat-Sprague	-Dawley - [rat]-Female					
Chemical:	Diethylhexyl	Phthalate- Parent compound					
HERO ID:	2000935						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality						
	Metric 1:	Reporting Quality	High	All critical and important information was reported. Reported information included information on the test substance (name and source, purity, CASRN), the test model (species, strain, sex, source, initial body weights, and animal parity), animal husbandry details (food and water availability, temperature, humidity, and light cycle), number per cage, exposure methods, experimental design, endpoint evaluations, and presentation of results. Starting age was not reported, however as the animal life stage is implied by the animal's parity, this deficiency does not have a negative impact on the quality of the study.			
Domain 2: Selection and	d Performance						
Domain 2. Selection and	Metric 2:	Allocation	Medium	Dams were randomly assigned to treatment groups by stratified randomization. The authors reported that mean body weights at GD0 did not differ among treatment groups. It was not specified how dams or litters were selected for the various outcomes assessed, or how fetuses were selected for measurement of testosterone production.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcome was measured using a standard laboratory kit.			
Domain 3: Confounding	r / Variable Cor	atral					
	Metric 4:	Confounding / Variable Control	Medium	Negative vehicle (olive oil) controls were included and gave the expected results. The study did not report taking measures to minimise the exposure to other plasticizers. Animals were housed in polycarbonate cages. Food, tap water, and bedding were not tested for contaminates, and the materials used to dispense water to the animals were not specified. The presence of contaminants could impact the study results; however, housing and husbandry conditions appear to be consistent across groups. Consistency of other potentially confounding factors (e.g., body weight, food or water intake) was not reported.			
Domain 4: Selective Re	porting and Att	rition					
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Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

HERO ID: 2000935 Table: 1 of 1

		con	tinued from p	revious page			
Study Citation: Health Outcome(s) and Reported	Saillenfait, and testoste Reproductiv	Saillenfait, A. M., Sabaté, J. P., Robert, A., Rouiller-Fabre, V., Roudot, A. C., Moison, D., Denis, F. (2013). Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. Journal of Applied Toxicology 33(9):1027-1035. Reproductive/Developmental-Fetal ex vivo testosterone production.					
Health Effect(s): Duration and Exposure Route:	Oral-Gavag	e-Duration: Reproductive/Developmental-	-1-F0 - gestatio	on (GD12-19)			
Species: Chemical: HERO ID:	Rat-Sprague Diethylhexy 2000935	e-Dawley - [rat]-Female /l Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	Medium	Insufficient information was provided to evaluate attrition or selective reporting. The to- tal number of dams initially included in each test group was not specified. The methods indicated the endpoint was assessed using both testes from 3 male fetuses from 8-12 lit- ters/dose group. The figure showing results specifies that the data were from 16 controls and 8 litters per treatment group. It is unclear whether any attrition occurred in any other litters.			
Domain 5: Exposure M	lethods Sensitiv	vity					
	Metric 6:	Chemical administration and characterization	Medium	The test material source and purity (>98%) were reported and the identity and purity of the test substance were confirmed using GC/MS. The test substance stability and storage were reported. Details of the preparation of the test solutions were not provided. An olive oil vehicle was used. Animals were dosed via gavage, the gavage volume (5 mL/kg) was appropriate. Doses were adjusted every three days based on dam body weights (daily would be preferred). Analytical measurements were not made			
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD 12-19. This exposure covers the critical period of male reproductive tract differentiation in rats.			
Domain 6: Outcome M	easures and Re	esults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	There are no major concerns regarding the specificity and validity of the protocol for measuring testosterone production. Testosterone production was measured individually ex vivo from both testes from 3 fetuses/litter (6 samples per litter), and tests were conducted on 8-12 litters per group (data reported for 16 control litters and 8 litters per treatment group). The assay used a 3-hr incubation period and testosterone levels were measured using TFC-MS/MS. The limit of quantitation was specified. The study tested two doses, using DEHP as a positive control for dose-response assays on a different phthalate. Since the authors justified their dose group spacing by using DEHP as a positive control, effects were observed at both doses of DEHP examined and a NOAEL for this endpoint cannot be determined. The test species and strain were appropriate for the study type.			
	Metric 9:	Results presentation	High	Results were reported in a figure (bar graph) showing means \pm SD. Statistical significance and sample sizes were noted. The litter was used as the experimental unit. Individual animal data were not provided.			
Additional Comments:	Only ex-viv	o testicular testosterone was evaluated for	data quality.				
Overall Quali	ty Deteri	mination	High				

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Diethylhexyl Phthalate

HERO ID: 2000935 Table: 1 of 1

		continued from previous page	
Study Citation:	Saillenfait, A. M., Sabaté, J. P., Robert, A., Ro and testosterone production in fetal rat testis a	ouiller-Fabre, V., Roudot, A. C., Mo after exposure to di-n-hexyl phthalat	ison, D., Denis, F. (2013). Dose-dependent alterations in gene expression e. Journal of Applied Toxicology 33(9):1027-1035.
Health Outcome(s)	Reproductive/Developmental-Fetal ex vivo te	stosterone production.	
and Reported			
Health Effect(s):			
Duration and	Oral-Gavage-Duration: Reproductive/Develop	pmental-1-F0 - gestation (GD12-19)	
Exposure Route:			
Species:	Rat-Sprague-Dawley - [rat]-Female		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	2000935		
Domain	Metric	Rating	Comments

Study Citation:	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2	-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and
	Chemical Toxicology 40(10):1499-1506.	
Health Outcome(s)	Reproductive/Developmental-Repro - mating and fertility (number of females	pregnant, number of females that delivered, number of litters/group. number
and Reported	of offspring, average litter size, average litter weight); Dev - survival Index, 1	ive pup body weight, sex ratio, neurobehavioral endpoints surface righting
Health Effect(s):	and negative geotaxis tests (PNDs 4 and 7); cliff avoidance was tested (PND exploratory behavior (week 3 and 8 weeks); and water T-maze test (week 7), be	7); swimming behavior (PNDs 4 and 14); olfactory orientation (PND 14); ody weight and food intake after weaning.
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)	-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1-
Exposure Route:	post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal	
Species:	Mouse-CD-1 - [mouse]-Both	
Chemical:	Diethylhexyl Phthalate- Parent compound	
HERO ID:	732820	
Domain	Matria	Commonto

Domani	wiethe	Kating	Comments
Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Medium	The test substance was identified by name (bis(2- ethylhexyl) phthalate (DEHP), a CASRN was not provided). The source and purity (>97%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins (4 weeks old at purchase). Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, number of animals/cage) were reported. Cage type and bedding type were reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as % in food and estimated based on food take. The duration was reported. Target, but not analytical concentrations in the food were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance	2		
Metric 2:	Allocation	Low	Allocation methods were not reported for F0 animals. One male and female were ran- domly selected from each F1 litter, but the method of allocation was not provided.
Metric 3:	Observational Bias / Blinding Changes	Low	Blinding was not reported. Some endpoints were not subjective in nature and did not require blinding (body weight, litter size, etc.). However, neurobehavioral endpoints are subjective in nature and should be evaluated blindly.
Domain 3: Confounding / Variable Co	ontrol		
Metric 4:	Confounding / Variable Control	Medium	Average food intake was reported for all periods (premating, mating, gestation, lactation and F1 generation) and was similar between the groups; suggesting palatability was not an issue. Body weights of maternal animals were not shown, but were reported to be similar to controls during preconception and mating and "the average body weight of dams showed no significant adverse effects during the gestation and lactation periods". A negative control group was included (basal diet) and responses were appropriate. Housing conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.

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Diethylhexyl Phthalate

		cont	inued from previ	ous page	
Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506. Reproductive/Developmental-Repro - mating and fertility (number of females pregnant, number of females that delivered, number of litters/group. number of offspring, average litter size, average litter weight); Dev - survival Index, live pup body weight, sex ratio, neurobehavioral endpoints surface righting and negative geotaxis tests (PNDs 4 and 7); cliff avoidance was tested (PND 7); swimming behavior (PNDs 4 and 14); olfactory orientation (PND 14); exploratory behavior (week 3 and 8 weeks); and water T-maze test (week 7), body weight and food intake after weaning. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 732820				
Domain	porting and Att	Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes. Table footnotes did not specify the number of animals included in each group for analysis, it is unclear if all animals were evaluated and included in analysis.	
Domain 5: Exposure M	thods Sensitiv	ity.			
Domain 5. Exposure inc	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Purity was not independently verified by the performing laboratory. The basal diet was mixed with 0.01, 0.03, and 0.09% of DEHP and formed into pellets. No additional details were provided on preparation or storage of the test material. Target test concentrations in food (%) were reported; there is no indication that analysis was done. The authors calculated dose equivalents in mg/kg/day for all groups; however, it was not specified how these calculations were made.	
	Metric 7:	Exposure timing, frequency, and duration	High	F0 animals were exposed for 4 weeks prior to mating, during mating and through lac- tation. This agrees with OECD 422 guidelines. Exposure was consistent across study groups. Groups were treated concurrently. Dose rationale was based on several other reproductive and developmental studies previously performed using DEHP.	
Domain 6: Outcome Me	asures and Res	sulte Dieplay			
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The animal species was appropriate. The number of ani- mals/group was appropriate as group sizes were large enough to yield at least 8 pregnant females/group per OECD 422 guidance. Sample sizes were sufficient to allow for sta- tistical analysis. The number of dose groups and dose spacing was adequate. Outcome methodologies for the F0 and F1 generations were adequately reported and sensitive for the endpoints assessed.	
	Metric 9:	Results presentation	Low	Results for some reproductive/developmental endpoints were shown in tables (shown as means \pm SD), however other endpoints were reported as negative in text (data not shown). Statistical analysis methods were reported and statistical significance was noted in tables. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided. As noted by Dishaw et al., 2020, the presentation of offspring data as means of individual animals, rather than as litter means, has the potential to overestimate the statistical significance of experimental findings.	

Additional Comments: None

Continued on next page ...

Diethylhexyl Phthalate

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HERO ID: 732820 Table: 1 of 3

		continued from previous page	
Study Citation:	Tanaka, T. (2002). Reproductive and neurob	behavioural toxicity study of bis(2-ethylho	exyl) phthalate (DEHP) administered to mice in the diet. Food and
	Chemical Toxicology 40(10):1499-1506.		
Health Outcome(s)	Reproductive/Developmental-Repro - mating	g and fertility (number of females pregnan	t, number of females that delivered, number of litters/group. number
and Reported	of offspring, average litter size, average litte	er weight); Dev - survival Index, live pup	body weight, sex ratio, neurobehavioral endpoints surface righting
Health Effect(s):	and negative geotaxis tests (PNDs 4 and 7);	cliff avoidance was tested (PND 7); swit	mming behavior (PNDs 4 and 14); olfactory orientation (PND 14);
	exploratory behavior (week 3 and 8 weeks);	and water T-maze test (week 7), body wei	ght and food intake after weaning.
Duration and	Oral-Diet-Duration: Reproductive/Developm	nental-1-F0- premating (4 weeks)-F0- ma	ating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1-
Exposure Route:	post-natal-F0- premating (4 weeks)-F0- mati	ng (5 days)-F1- post-natal	
Species:	Mouse-CD-1 - [mouse]-Both		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	732820		
Domain	Metric	Rating	Comments
Overall Ouali	ty Determination	Medium	

Study Citation: Health Outcome(s) and Reported	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506. Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-De post-natal-Fe Mouse-CD- Diethylhexy 732820	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 732820			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name (bis(2- ethylhexyl) phthalate (DEHP), a CASRN was not provided). The source and purity (>97%) were reported. Test ani- mal species, strain, sex, age, and source were reported. It was not specified if mice were virgins (4 weeks old at purchase). Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, number of animals/cage) were reported. Cage type and bedding type were reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as % in food and estimated based on food take. The duration was reported. Target, but not analytical concentrations in the food were reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Low	Allocation methods were not reported for F0 animals.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	BW and food intake: Blinding was not reported however endpoints were not subjective in nature (body weight and food intake). Neuro: Blinding was not reported however exploratory behavior was assessed in a non-subjective manner using detectors of near- infrared photosensors for measuring spontaneous motor activity.	
Domain 3: Confounding	a / Variable Co	atrol			
	Metric 4:	Confounding / Variable Control	Medium	Average food intake was reported for all periods (premating, mating, gestation, lactation and F1 generation) and was similar between the groups; suggesting palatability was not an issue. Body weights of maternal animals were not shown, but were reported to be similar to controls during preconception and mating and "the average body weight of dams showed no significant adverse effects during the gestation and lactation periods". A negative control group was included (basal diet) and responses were appropriate. Housing conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.	
Domain 4: Selective Re	porting and At	rition			
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes. Table footnotes did not specify the number of animals included in each group for analysis, it is unclear if all animals were evaluated and included in analysis.	
Continued on next page					

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		conti	inued from previ	ous page		
Study Citation:	Tanaka, T. (Chemical To	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicalogy 40(10):1499, 1506				
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior					
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-D post-natal-F Mouse-CD- Diethylhexy 732820	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 732820				
Domain		Metric	Rating	Comments		
Domain 5: Exposure M	ethods Sensitiv	/ity	-			
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Purity was not independently verified by the performing laboratory. The basal diet was mixed with 0.01, 0.03, and 0.09% of DEHP and formed into pellets. No additional details were provided on preparation or storage of the test material. Target test concentrations in food (%) were reported; there is no indication that analysis was done. The authors calculated dose equivalents in mg/kg/day for all groups; however, it was not specified how these calculations were made.		
	Metric 7:	Exposure timing, frequency, and duration	High	F0 animals were exposed for 4 weeks prior to mating, during mating and through lac- tation. This agrees with OECD 422 guidelines. Exposure was consistent across study groups. Groups were treated concurrently. Dose rationale was based on several other reproductive and developmental studies previously performed using DEHP.		
Domain 6: Outcome Me	easures and Re	sults Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The animal species was appropriate. The number of ani- mals/group was appropriate as group sizes were large enough to yield at least 8 pregnant females/group per OECD 422 guidance. Sample sizes were sufficient to allow for sta- tistical analysis. The number of dose groups and dose spacing was adequate. Outcome methodologies were adequately reported and sensitive for the endpoints assessed.		
	Metric 9:	Results presentation	Medium	Food intake was reported in Table as mean intake +/- SD. Body weights and exploratory behavior effects were reported as negative in text (data not shown). Statistical analysis methods were reported and appropriate.		
Additional Comments:	None					
Overall Quali	ty Deteri	nination	Medium			

Study Citation: Health Outcome(s) and Reported	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506. Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior				
Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-De post-natal-Fe Mouse-CD- Diethylhexy 732820	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 732820			
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Medium	The test substance was identified by name (bis(2- ethylhexyl) phthalate (DEHP), a CASRN was not provided). The source and purity (>97%) were reported. Test ani- mal species, strain, sex, age, and source were reported. It was not specified if mice were virgins (4 weeks old at purchase). Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, number of animals/cage) were reported. Cage type and bedding type were reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as % in food and estimated based on food take. The duration was reported. Target, but not analytical concentrations in the food were reported. Endpoint evaluation methods were reported along with quantitative data.	
Domain 2: Selection and	d Performance				
	Metric 2:	Allocation	Low	Allocation methods were not reported for F0 animals.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	BW and food intake: Blinding was not reported however endpoints were not subjective in nature (body weight and food intake). Neuro: Blinding was not reported however exploratory behavior was assessed in a non-subjective manner using detectors of near- infrared photosensors for measuring spontaneous motor activity.	
Domain 3: Confounding	a / Variable Co	atrol			
	Metric 4:	Confounding / Variable Control	Medium	Average food intake was reported for all periods (premating, mating, gestation, lactation and F1 generation) and was similar between the groups; suggesting palatability was not an issue. Body weights of maternal animals were not shown, but were reported to be similar to controls during preconception and mating and "the average body weight of dams showed no significant adverse effects during the gestation and lactation periods". A negative control group was included (basal diet) and responses were appropriate. Housing conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.	
Domain 4: Selective Re	porting and At	rition			
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes. Table footnotes did not specify the number of animals included in each group for analysis, it is unclear if all animals were evaluated and included in analysis.	
Continued on next page					

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		conti	inued from previ	ous page			
Study Citation:	Tanaka, T. (Chemical T	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506					
Health Outcome(s) and Reported Health Effect(s):	Nutritional/	Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior					
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal Mouse-CD-1 - [mouse]-Both Diethylhexyl Phthalate- Parent compound 732820						
Domain		Metric	Rating	Comments			
Domain 5: Exposure M	lethods Sensitiv	vity					
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Purity was not independently verified by the performing laboratory. The basal diet was mixed with 0.01, 0.03, and 0.09% of DEHP and formed into pellets. No additional details were provided on preparation or storage of the test material. Target test concentrations in food (%) were reported; there is no indication that analysis was done. The authors calculated dose equivalents in mg/kg/day for all groups; however, it was not specified how these calculations were made.			
	Metric 7:	Exposure timing, frequency, and duration	High	F0 animals were exposed for 4 weeks prior to mating, during mating and through lac- tation. This agrees with OECD 422 guidelines. Exposure was consistent across study groups. Groups were treated concurrently. Dose rationale was based on several other reproductive and developmental studies previously performed using DEHP.			
Domain 6: Outcome M	easures and Re	sults Display					
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The animal species was appropriate. The number of ani- mals/group was appropriate as group sizes were large enough to yield at least 8 pregnant females/group per OECD 422 guidance. Sample sizes were sufficient to allow for sta- tistical analysis. The number of dose groups and dose spacing was adequate. Outcome methodologies were adequately reported and sensitive for the endpoints assessed.			
	Metric 9:	Results presentation	Medium	Food intake was reported in Table as mean intake +/- SD. Body weights and exploratory behavior effects were reported as negative in text (data not shown). Statistical analysis methods were reported and appropriate.			
Additional Comments:	None						
Overall Quali	ty Deteri	nination	Medium				

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmen-
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments.
Health Effect(s):	Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross
Duration and	observations. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-
Exposure Route:	lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric	Rating	Comments		
Domain 1: Reporting Quality					
Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.		
Domain 2: Selection and Performance					
Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups.		
Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for the main study, but the outcomes of interest were not subjective in nature, or blinding was not required.		
Domain 3: Confounding / Variable Cor	ntrol				
Metric 4:	Confounding / Variable Control	Medium	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A positive control is not required for the study type. The control responses were appropriate. There were no differences in food intake (g/animal/day) across groups prior to and throughout mating or gestation. Feed intake (g/kg BW) in high-dose males was significantly decreased in the absence of body weight changes. Food and water consumption in dams was decreased in a dose-related manner during lactation, but no differences in final body weights were observed. It is unclear if the decreases were related to palatability issues.		
Domain 4: Selective Perperting and Att	rition				
Metric 5:	Selective Reporting and Attrition	High	No adults died and data were reported for all specified outcomes. There was no indica- tion of issues with animal attrition or selective reporting.		
Domain 5: Exposure Methods Sensitiv	ity				
	Continued on next page				

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Health Outcome(s) Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmental parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. and Reported Health Effect(s): Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-**Duration and Exposure Route:** lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Me	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. The number of exposure groups and spacing for this range-finding study were based on information from other studies on this chemical of interest. The endpoints evaluated were appropriate for a range-finding study. Sample sizes were specified and were adequate. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 8 mating pairs.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			
Overall Quali	ty Deterr	nination	High	

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmen-
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments.
Health Effect(s):	Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis
	decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross
Duration and	observations. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-
Exposure Route:	lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Quality	r				
Me	etric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and Perf	formance				
Me	etric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups.	
Me	etric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for the main study, but the outcomes of interest were not subjective in nature, or blinding was not required.	
Domain 3: Confounding / Var	riable Con	trol			
Me	etric 4:	Confounding / Variable Control	Medium	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A positive control is not required for the study type. The control responses were appropriate. There were no differences in food intake (g/animal/day) across groups prior to and throughout mating or gestation. Feed intake (g/kg BW) in high-dose males was significantly decreased in the absence of body weight changes. Food and water consumption in dams was decreased in a dose-related manner during lactation, but no differences in final body weights were observed. It is unclear if the decreases were related to palatability issues.	
Domain 4: Salaative Reporting and Attrition					
Me	etric 5:	Selective Reporting and Attrition	High	No adults died and data were reported for all specified outcomes. There was no indica- tion of issues with animal attrition or selective reporting.	
Domain 5: Exposure Methods Sensitivity					
Continued on next page					

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Health Outcome(s) Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmental parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. and Reported Health Effect(s): Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-**Duration and Exposure Route:** lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Me	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. The number of exposure groups and spacing for this range-finding study were based on in- formation from other studies on this chemical of interest. The endpoints evaluated were appropriate for a range-finding study. Sample sizes were specified and were adequate. Generally, for reproduction studies, enough mating pairs to generate 20 litters is pre- ferred. This study only used 8 mating pairs.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			
Overall Quality Determination High			High	

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Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmen-
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments.
Health Effect(s):	Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis
	decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross
Duration and	observations. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-
Exposure Route:	lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Quality	r				
Me	etric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and Perf	formance				
Me	etric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups.	
Me	etric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for the main study, but the outcomes of interest were not subjective in nature, or blinding was not required.	
Domain 3: Confounding / Var	riable Con	trol			
Me	etric 4:	Confounding / Variable Control	Medium	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A positive control is not required for the study type. The control responses were appropriate. There were no differences in food intake (g/animal/day) across groups prior to and throughout mating or gestation. Feed intake (g/kg BW) in high-dose males was significantly decreased in the absence of body weight changes. Food and water consumption in dams was decreased in a dose-related manner during lactation, but no differences in final body weights were observed. It is unclear if the decreases were related to palatability issues.	
Domain 4: Salaative Reporting and Attrition					
Me	etric 5:	Selective Reporting and Attrition	High	No adults died and data were reported for all specified outcomes. There was no indica- tion of issues with animal attrition or selective reporting.	
Domain 5: Exposure Methods Sensitivity					
Continued on next page					

May 2025

Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Health Outcome(s) Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmental parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. and Reported Health Effect(s): Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations. Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-**Duration and Exposure Route:** lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Me	easures and Res	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. The number of exposure groups and spacing for this range-finding study were based on information from other studies on this chemical of interest. The endpoints evaluated were appropriate for a range-finding study. Sample sizes were specified and were adequate. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 8 mating pairs.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			
Overall Quality Determination High			High	

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Study Citation:	TherImmune Research Corporation, (2004).	Diethylhexylphthalate: Multigener	ational reproductive assessment by continuous breeding when administered
-	to Sprague-Dawley rats in the diet: Final repo	ort.	
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-A	dult body weights food and water	consumption-Reproductive/Developmental-Reproductive and developmen-
and Deported	tal nonometers from EQ (E1a, E1b, E1a), E1	$(E_{2}^{2}, E_{2}^{2}) = E_{2}^{2} (E_{2}^{2}, E_{2}^{2}) = E_{2}^{2} (E_{2}^{2}) = E_{2}^{2} (E_{2}^{2}) = E_{2}^{2} ($	(itters) and from E1a and E2a grossover mating experimenta
and Reported		(F2a, F2b, F2c), F2 (F5a, F5b, F3	c) manings (muers), and from Fic and Fic crossover mating experiments.
Health Effect(s):	Endpoints include reproductive performance,	standard litter parameters, growth	and reproductive development of offspring (e.g., preputial separation, testis
	decent, vaginal opening), and reproductive tr	act malformations (RTMs). Repro	ductive organ weights of adults, estrous cyclicity, sperm parameters, gross
	observationsHepatic/Liver-Liver weights, g	ross necropsy, histopathology-Ren	al/Kidney-Kidney weights, gross necropsy, histopathology (including the
	bladder)-Other (please specify below) (Endo	crine)-Adrenals and pituitary organ	weights, gross necropsy, histopathology
Duration and	Oral-Diet-Duration: Reproductive/Developm	nental-3-F0- premating (6 weeks)-	F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21	to PND81))-F1- mating (9 weeks	cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premat	ting (~60 days (PND21 to PND81)	-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through r	necropsy)-F0- premating (6 weeks	-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through	necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning	through necropsy)	
Species:	Rat-Sprague-Dawley - [rat]-Both	unough neeropsy)	
Chemical.	Diethylbeyyl Phthalate- Parent compound		
	2108000 Limber d UEDO ID(-), 2108000 122	AE15 555((Q5	
HEKU ID:	5108900 Linked HERO ID(\$): 3108900, 133	4313, 3330083	
Domain	Metric	Rating	Comments

			8	
Domain 1: Reporting Quality				
	ric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Perfo	ormance			
Metr	ric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
Metr	ric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confounding / Vari	iable Cor	atrol		
Metr	ric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 1: Selective Deporting	g and Att	rition		
Metr	ric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."
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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. **Health Outcome(s)** Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmenand Reported tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis Health Effect(s): decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations.-Hepatic/Liver-Liver weights, gross necropsy, histopathology-Renal/Kidney-Kidney weights, gross necropsy, histopathology (including the bladder)-Other (please specify below) (Endocrine)-Adrenals and pituitary organ weights, gross necropsy, histopathology **Duration and** Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation **Exposure Route:** (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- postnatal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685 Domain Metric Comments Rating

Domain 5: Exposure Me	ethods Sensitiv	ity		
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Me	asures and Res	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). D	iethylhexylphthalate: Multigener	rational reproductive assessment by continuous breeding when administered				
	to Sprague-Dawley rats in the diet: Final report	t.					
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-Ad	ult body weights, food and water	consumption-Reproductive/Developmental-Reproductive and developmen-				
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F2	3c) matings (litters), and from F1c and F2c crossover mating experiments.				
Health Effect(s):	Endpoints include reproductive performance, s	tandard litter parameters, growth	and reproductive development of offspring (e.g., preputial separation, testis				
	decent, vaginal opening), and reproductive tra	ct malformations (RTMs). Repro	oductive organ weights of adults, estrous cyclicity, sperm parameters, gross				
	observationsHepatic/Liver-Liver weights, groups	oss necropsy, histopathology-Rea	nal/Kidney-Kidney weights, gross necropsy, histopathology (including the				
	bladder)-Other (please specify below) (Endocr	ine)-Adrenals and pituitary organ	n weights, gross necropsy, histopathology				
Duration and	Oral-Diet-Duration: Reproductive/Developme	ntal-3-F0- premating (6 weeks)-	F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation				
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 t	o PND81))-F1- mating (9 weeks	s cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-				
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation						
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to						
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9						
	weeks cohabitation)-F2- post-natal (weaning the	hrough necropsy)					
Species:	Rat-Sprague-Dawley - [rat]-Both						
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334	515, 5556685					
Domain	Metric	Rating	Comments				
Domain 1: Reporting Q	uality						

Domain 1: Reporting	Quality			
	Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection a	nd Performance			
	Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
	Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confoundi	ng / Variable Co	ntrol		
Domain 5. Comoundi	Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 4: Selective R	eporting and At	trition		
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."

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Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Health Outcome(s) Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmenand Reported tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis Health Effect(s): decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations.-Hepatic/Liver-Liver weights, gross necropsy, histopathology-Renal/Kidney-Kidney weights, gross necropsy, histopathology (including the bladder)-Other (please specify below) (Endocrine)-Adrenals and pituitary organ weights, gross necropsy, histopathology **Duration and** Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation **Exposure Route:** (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- postnatal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685 Domain Metric Comments Rating

Domain 5: Exposure Me	thods Sensitivi	ty		
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Me	asures and Res	ults Display		
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). I	Diethylhexylphthalate: Multigener	ational reproductive assessment by continuous breeding when administered				
	to Sprague-Dawley rats in the diet: Final repo	ort.					
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-Ad	dult body weights, food and water	consumption-Reproductive/Developmental-Reproductive and developmen-				
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1	(F2a, F2b, F2c), F2 (F3a, F3b, F3	Bc) matings (litters), and from F1c and F2c crossover mating experiments.				
Health Effect(s):	Endpoints include reproductive performance,	standard litter parameters, growth	and reproductive development of offspring (e.g., preputial separation, testis				
	decent, vaginal opening), and reproductive tra	act malformations (RTMs). Repro	ductive organ weights of adults, estrous cyclicity, sperm parameters, gross				
	observationsHepatic/Liver-Liver weights, g	ross necropsy, histopathology-Rer	nal/Kidney-Kidney weights, gross necropsy, histopathology (including the				
	bladder)-Other (please specify below) (Endoc	crine)-Adrenals and pituitary organ	weights, gross necropsy, histopathology				
Duration and	Oral-Diet-Duration: Reproductive/Developm	ental-3-F0- premating (6 weeks)-l	F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation				
Exposure Route:	(21 days)-F1- premating (~60 days (PND21	to PND81))-F1- mating (9 weeks	cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-				
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation						
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to						
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9						
	weeks cohabitation)-F2- post-natal (weaning	through necropsy)					
Species:	Rat-Sprague-Dawley - [rat]-Both						
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334	4515, 5556685					
Domain	Metric	Rating	Comments				
Domain 1: Reporting Q	uality						

Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Performance			
Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confounding / Variable Co	antrol		
Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 4: Selective Reporting and A	ttrition		
Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."

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Domain 5: Exposure Method	ds Sensitivi	ty		
М	etric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
М	letric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measur	res and Res	ults Display		
Μ	etric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.
М	letric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments: N	one			

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). I	Diethylhexylphthalate: Multigene	ational reproductive assessment by continuous breeding when administered
-	to Sprague-Dawley rats in the diet. Final repo	ort C	
Hoalth Outcomo(s)	Mortality Mortality Nutritional/Metabolic A	dult body weights food and water	concumption Penroductive/Developmental Penroductive and developmen
Inearth Outcome(s)	Wortanty-Wortanty-Nutritional/Wetabolie-A		consumption-reproductive/Developmental-reproductive and development-
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1	(F2a, F2b, F2c), F2 (F3a, F3b, F	3c) matings (litters), and from F1c and F2c crossover mating experiments.
Health Effect(s):	Endpoints include reproductive performance,	standard litter parameters, growth	and reproductive development of offspring (e.g., preputial separation, testis
	decent, vaginal opening), and reproductive tr	act malformations (RTMs). Repro	oductive organ weights of adults, estrous cyclicity, sperm parameters, gross
	observationsHepatic/Liver-Liver weights, g	ross necropsy, histopathology-Re	nal/Kidney-Kidney weights, gross necropsy, histopathology (including the
	bladder)-Other (please specify below) (Endoo	rine)-Adrenals and pituitary orga	n weights, gross necropsy, histopathology
Duration and	Oral-Diet-Duration: Reproductive/Developm	ental-3-F0- premating (6 weeks)-	F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21	to PND81))-F1- mating (9 weeks	s cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premat	ing (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through n	ecropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)	-F1- post-natal (weaning through	n necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning	through necropsy)	
Species:	Rat-Sprague-Dawley - [rat]-Both		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	3108900 Linked HERO ID(s): 3108900, 133	4515, 5556685	
Domain	Metric	Rating	Comments

Domani	with	Kating	Comments
Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Performan	ce		
Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confounding / Variable C	Control		
Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 4: Selective Reporting and	Attrition		
Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Health Outcome(s) Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmenand Reported tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis Health Effect(s): decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations.-Hepatic/Liver-Liver weights, gross necropsy, histopathology-Renal/Kidney-Kidney weights, gross necropsy, histopathology (including the bladder)-Other (please specify below) (Endocrine)-Adrenals and pituitary organ weights, gross necropsy, histopathology **Duration and** Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation **Exposure Route:** (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- postnatal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685 Domain Metric Comments Rating

Domain 5: Exposure Methods Sensitiv	ity		
Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Res	sults Display		
Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.
Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments: None			

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004).	Diethylhexylphthalate: Multigenet	ational reproductive assessment by continuous breeding when administered
Study Churchin	to Sprague-Dawley rats in the diet: Final repo	ort.	
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-A	dult body weights, food and water	consumption-Reproductive/Developmental-Reproductive and developmen-
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1	(F2a, F2b, F2c), F2 (F3a, F3b, F3b, F3b, F3b, F3b, F3b, F3b, F3b	Bc) matings (litters), and from F1c and F2c crossover mating experiments.
Health Effect(s):	Endpoints include reproductive performance,	standard litter parameters, growth	and reproductive development of offspring (e.g., preputial separation, testis
	decent, vaginal opening), and reproductive tr	act malformations (RTMs). Repro	ductive organ weights of adults, estrous cyclicity, sperm parameters, gross
	observationsHepatic/Liver-Liver weights, g	ross necropsy, histopathology-Re	nal/Kidney-Kidney weights, gross necropsy, histopathology (including the
	bladder)-Other (please specify below) (Endo	crine)-Adrenals and pituitary organ	n weights, gross necropsy, histopathology
Duration and	Oral-Diet-Duration: Reproductive/Developm	nental-3-F0- premating (6 weeks)-	F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21	to PND81))-F1- mating (9 weeks	cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premat	ting (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through n	ecropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation))-F1- post-natal (weaning through	necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning	through necropsy)	
Species:	Rat-Sprague-Dawley - [rat]-Both		
Chemical:	Diethylhexyl Phthalate- Parent compound		
HERO ID:	3108900 Linked HERO ID(s): 3108900, 133	4515, 5556685	
Domain	Metric	Rating	Comments

Domain		wiethe	Kating	Comments		
Domain 1: Reporting Quality						
	Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.		
Domain 2: Selection and F	Performance					
	Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).		
:	Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.		
Domain 3: Confounding /	Variable Con	trol				
	Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.		
Domain 4. Salastiva Dara	uting and Atte	itian.				
Domain 4: Selective Repo	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."		

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

... continued from previous page **Study Citation:** TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Health Outcome(s) Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmenand Reported tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis Health Effect(s): decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations.-Hepatic/Liver-Liver weights, gross necropsy, histopathology-Renal/Kidney-Kidney weights, gross necropsy, histopathology (including the bladder)-Other (please specify below) (Endocrine)-Adrenals and pituitary organ weights, gross necropsy, histopathology **Duration and** Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation **Exposure Route:** (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- postnatal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685 Domain Metric Comments Rating

Domain 5: Exposure Me	ethods Sensitivi	ity					
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.			
Domain 6: Outcome Me	Domain 6: Outcome Measures and Results Display						
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.			
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.			
Additional Comments:	None						

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). D	iethylhexylphthalate: Multigener	rational reproductive assessment by continuous breeding when administered				
	to Sprague-Dawley rats in the diet: Final report	t.					
Health Outcome(s)	Mortality-Mortality-Nutritional/Metabolic-Ad	ult body weights, food and water	consumption-Reproductive/Developmental-Reproductive and developmen-				
and Reported	tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F2	3c) matings (litters), and from F1c and F2c crossover mating experiments.				
Health Effect(s):	Endpoints include reproductive performance, s	tandard litter parameters, growth	and reproductive development of offspring (e.g., preputial separation, testis				
	decent, vaginal opening), and reproductive tra	ct malformations (RTMs). Repro	oductive organ weights of adults, estrous cyclicity, sperm parameters, gross				
	observationsHepatic/Liver-Liver weights, groups	oss necropsy, histopathology-Rea	nal/Kidney-Kidney weights, gross necropsy, histopathology (including the				
	bladder)-Other (please specify below) (Endocr	ine)-Adrenals and pituitary organ	n weights, gross necropsy, histopathology				
Duration and	Oral-Diet-Duration: Reproductive/Developme	ntal-3-F0- premating (6 weeks)-	F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation				
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 t	o PND81))-F1- mating (9 weeks	s cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-				
	natal (weaning through necropsy)-F2- premating	ng (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation				
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to						
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9						
	weeks cohabitation)-F2- post-natal (weaning the	hrough necropsy)					
Species:	Rat-Sprague-Dawley - [rat]-Both						
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334	515, 5556685					
Domain	Metric	Rating	Comments				
Domain 1: Reporting Q	uality						

Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Performance			
Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confounding / Variable Co	antrol		
Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 4: Selective Reporting and A	ttrition		
Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."

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Human Health Hazard Animal Toxicology Evaluation

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Diethylhexyl Phthalate

Study Citation:

Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	to Sprague-Dawley rats in the diet: Final report. Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmen- tal parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observationsHepatic/Liver-Liver weights, gross necropsy, histopathology-Renal/Kidney-Kidney weights, gross necropsy, histopathology (including the bladder)-Other (please specify below) (Endocrine)-Adrenals and pituitary organ weights, gross necropsy, histopathology Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F1- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound					
Domain		Metric	Rating	Comments		
Domain 5: Exposure M	ethods Sensitiv	ity	Ruting	Comments		
·	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.		
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.		
Domain 6: Outcome M	easures and Re	sulte Dieplay				
Domain 0. Outcome Mi	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.		
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.		
Additional Comments:	None					
Overall Quali	ty Detern	nination	High			

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any
	gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Quality					
Metr	ric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and Perfo	ormance				
Metr	ric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).	
Met	ric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.	
Domain 3: Confounding / Vari	able Con	trol			
Met	ric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.	
Domain 4: Selective Reporting	g and Att	rition			
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HERO ID: 3108900 Table: 10 of 21

Study Citation:

Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species:	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid/Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues- Coral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F1- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1 - premating (9 we					
Chemical: HERO ID:	Diethylhexy 3108900 Lin	Phthalate- Parent compound nked HERO ID(s): 3108900, 1334515, 55	56685			
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."		
Domain 5: Exposure M	fethods Sensitiv Metric 6:	vity Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.		
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.		
Domain 6: Outcome N	leasures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.		
		Con	tinued on ne	xt page		

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 10 of 21

		•	continued from p	revious page			
Study Citation:	TherImmune	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered					
Health Outcome(s) and Reported Health Effect(s): Duration and	Inerimmune Research Corporation, (2004). Diethylnexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation						
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)						
Species:	Rat-Sprague	Kat-Sprague-Dawley - [rat]-Both					
UIEDO ID.	2108000 Lin	Diethylhexyl Phthalate- Parent compound					
HERO ID:	5108900 Lii	iked HERO ID(s). 5108900, 15545	15, 5550085				
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Koute:	(21 days)-F1- premating (~60 days (FND21 to FND31))-F1- mating (9 weeks contained)-F1 - gestation (~21 days)-F1- factation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments		
Domain 1: Reporting Quality						
Met	tric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.		
Domain 2: Selection and Perfe	ormance					
Met	tric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).		
Met	tric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.		
Domain 3: Confounding / Var	iable Cor	ntrol				
Met	tric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.		
Domain 4: Selective Reporting and Attrition						
Continued on next page						

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 11 of 21

Study Citation:	TherImmur	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered						
Health Outcome(s) and Reported Health Effect(s):	 Sprague-Dawley fats in the deft. Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Iumg/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues. Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F1- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2 - post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2 - lactation (21 days)-F2 - post-natal (weaning through necropsy)-F2 - premating (~60 days (PND21 to PND81))-F2 - mating (9 weeks cohabitation)-F1 - gest-nata							
Duration and Exposure Route: Species: Chemical:								
HERO ID:	3108900 Li	inked HERO ID(s): 3108900, 1334515, 555	56685					
Domain		Metric	Rating	Comments				
	Metric 5:	Selective Reporting and Attrition	Hıgh	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."				
Domain 5: Exposure	Methods Sensiti	vity						
Donkin et Exposure	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.				
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.				
Domain 6: Outcome l	Measures and Ro	esults Display						
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.				
		Cont	tinued on ne	xt page				

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 11 of 21

Diethylhexyl Phthalate

		••	continued from p	revious page		
Study Citation:	TherImmune	e Research Corporation, (2004). Die	thylhexylphthalate:	Multigenerational reproductive assessment by continuous breeding when administered		
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-					
Duration and Exposure Route: Species: Chemical: HERO ID:	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound					
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	High			

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any
	gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Quality					
Metr	ric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and Perfo	ormance				
Metr	ric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).	
Met	ric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.	
Domain 3: Confounding / Vari	able Con	trol			
Met	ric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.	
Domain 4: Selective Reporting and Attrition					
Continued on next page					

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

				Letters Lu2.		
Study Citation:	TherImmun	ne Research Corporation, (2004). Diethylhe	exylphthalate	Multigenerational reproductive assessment by continuous breeding when administered		
Health Outcome(s) and Reported Health Effect(s):	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-					
Duration and Exposure Route: Species:	Öral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both					
HERO ID:	3108900 Li	nked HERO ID(s): 3108900, 1334515, 555	56685			
Domain		Metric	Rating	Comments		
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."		
Domain 5: Exposure M	/lethods Sensiti Metric 6:	vity Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.		
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.		
Domain 6: Outcome N	leasures and Re	esults Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.		

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 12 of 21

			continued from p	revious page			
Study Citation:	TherImmun	e Research Corporation, (2004). D	iethylhexylphthalate:	Multigenerational reproductive assessment by continuous breeding when administered			
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues- Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues- Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues- language tissues.						
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound 3108000 Linkad HEPO LD(e): 3108000 1334515 5556685						
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	High				

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Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any
	gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Quality					
Metr	ric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and Perfo	ormance				
Metr	ric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).	
Met	ric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.	
Domain 3: Confounding / Vari	able Con	trol			
Met	ric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.	
Domain 4: Selective Reporting and Attrition					
Continued on next page					

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

Study Citation:	TherImmune	Research Corporation, (2004). Diethylhex	ylphthalate:	Multigenerational reproductive assessment by continuous breeding when administered			
Health Outcome(s) and Reported Health Effect(s):	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid/Parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respira						
Duration and	gross findings in related tissues Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation						
Exposure Route:	(21 days)-F1	- premating (~60 days (PND21 to PND81))-F1- matir	g (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- to PND(1)) F2 mating (9 weeks cohabitation) F2 gestation (~21 days) F2 lactation			
	(21 days)-F2	- post-natal (weaning through necropsy)-F2-	FO- prematin	g (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to			
	PND81))-F1-	mating (9 weeks cohabitation)-F1- post-	natal (wean	ing through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9			
Species:	Rat-Sprague-	Dawley - [rat]-Both	ecropsy)				
Chemical:	Diethylhexyl	Phthalate- Parent compound	5685				
Domain	5108900 Lill	Metric	Rating	Comments			
Domain	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."			
Domain 5: Exposure Me	thods Sensitivi	fxy					
Domain 5. Exposure we	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.			
Domain 6: Outcome Me	asures and Res	ults Display					
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.			

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Human Health Hazard Animal Toxicology Evaluation

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HERO ID: 3108900 Table: 13 of 21

Diethylhexyl Phthalate		

Study Citation:	TherImmune	Research Corporation, (2004). Diet	thylhexylphthalate:	Multigenerational reproductive assessment by continuous breeding when administered		
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-					
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation					
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F2- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9					
Species:	Rat-Sprague	-Dawley - [rat]-Both				
Chemical:	Diethylhexy	Phthalate- Parent compound				
HERO ID:	3108900 Lin	ked HERO ID(s): 3108900, 133451	5, 5556685			
Domain		Metric	Rating	Comments		
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.		

Additional Comments: None

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any
	gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	Domain 1: Reporting Quality						
	Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.			
Domain 2: Selection and	d Performance						
	Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).			
	Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.			
Domain 4: Selective Reporting and Attrition							
	Continued on next page						

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

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Study Citation:	TherImmune	Research Corporation, (2004). Diethylhex	ylphthalate:	Multigenerational reproductive assessment by continuous breeding when administered			
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues- Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues- Lung/Respiratory-Gross necropsy and histopathology if any gross findings if any gross gr						
Duration and Exposure Route:	gross findings in related tissues Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- post-natal (weaning through necropsy)						
Chemical:	Diethylhexyl	Phthalate- Parent compound					
HERO ID:	3108900 Lin	ked HERO ID(s): 3108900, 1334515, 5556	5685				
Domain		Metric	Rating	Comments			
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."			
Domain 5: Exposure Me	ethods Sensitivi Metric 6:	ty Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.			
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.			
Domain 6: Outcome Me	ocures and Dec	ulte Display					
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.			

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Human Health Hazard Animal Toxicology Evaluation

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HERO ID: 3108900 Table: 14 of 21

Diethylhexyl Phthalate		

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered					
	to Sprague-Dawley rats in the diet: Final report.	5 5 1				
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasion	ns and alopecia, re	ddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched			
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge					
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or	protruding eyes, o	pacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,			
	urine stains, few feces, ulcers, swelling, small sta	tionary tissue mass	, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,			
	malocclusion, papilloma, obesity-Thyroid-Thyro	oid/parathyroid gro	ss necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and			
	histopathology if any gross findings in related ti	ssues-Immune/Hen	hatological-Gross necropsy and histopathology if any gross findings in related tissues-			
	Lung/Respiratory-Gross necropsy and histopath	ology if any gross	findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any			
	gross findings in related tissues					
Duration and	Oral-Diet-Duration: Reproductive/Development	al-3-F0- premating	(6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation			
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to	PND81))-F1- matir	ng (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-			
	natal (weaning through necropsy)-F2- premating	(~60 days (PND21	to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation			
	(21 days)-F2- post-natal (weaning through necr	opsy)-F0- prematin	g (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to			
	PND81))-F1- mating (9 weeks cohabitation)-F1	- post-natal (wean	ing through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9			
~ .	weeks cohabitation)-F2- post-natal (weaning thr	ough necropsy)				
Species:	Kat-Sprague-Dawley - [rat]-Both					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685					
Domain	Metric	Rating	Comments			
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental			
			unit where appropriate. All data were quantitatively reported as incidences or means \pm			
			SE, and individual animal data were provided.			
Additional Comments:	None					

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any
	gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments
Domain 1: Reporting Quality				
Metr	ric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Perfo	ormance			
Metr	ric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
Met	ric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confounding / Vari	able Con	trol		
Met	ric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 4: Selective Reporting and Attrition				
Continued on next page				

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation ...continued from previous page

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report			
Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	to Sprague-L Skin/Connec posture, lang from the eye urine stains, malocclusion histopatholog Lung/Respira gross finding Oral-Diet-Du (21 days)-F1 natal (weanin (21 days)-F2 PND81))-F1 weeks cohab Rat-Sprague- Diethylhexyl 3108900 Lin	Jawley rats in the diet: Final report. tive Tissue-Clinical signs: abrasions and guid behavior, lethargy, tremors, prostratic s, lacrimation, squinting, bulging or protr few feces, ulcers, swelling, small stationar n, papilloma, obesity-Thyroid-Thyroid/par gy if any gross findings in related tissues- atory-Gross necropsy and histopathology is in related tissues iration: Reproductive/Developmental-3-F - premating (~60 days (PND21 to PND8 ng through necropsy)-F2- premating (~60 - post-natal (weaning through necropsy)- mating (9 weeks cohabitation)-F1- pos- itation)-F2- post-natal (weaning through r -Dawley - [rat]-Both Phthalate- Parent compound ked HERO ID(s): 3108900, 1334515, 555	l alopecia, re on; gross necr uding eyes, o y tissue mass rathyroid gros Immune/Hen if any gross 0- premating 1))-F1- matin days (PND21 -F0- prematin t-natal (wean hecropsy)	ddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched ropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge pacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, , paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, ss necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and hatological-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings in related tissues-findings in related tissues-findings in related tissues-findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-findings (9 weeks cohabitation)-F0 - gestation (~21 days)-F1 - post-to PND81))-F2- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F2 - mating (9 might be provid
Domain		Metric	Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."
Domain 5: Exposure Me	ethods Sensitivi	ity		
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were

Exposure timing, frequency, and High duration High advance of the study type. The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.

Domain 6: Outcome Measures and Results Display

Metric 7:

Metric 8: Endpoint sensitivity and specificity

Low The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 15 of 21

			continued from p	revious page			
Study Citation:	TherImmune	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered					
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs; urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-						
Exposure Route:	(21 days)-F1	1- premating (~60 days (PND21 to	o PND81))-F1- matin	g (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-			
Species: Chemical: HERO ID:	natal (weani (21 days)-F2 PND81))-F1 weeks cohab Rat-Sprague Diethylhexy 3108900 Lir	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.			
Additional Comments:	None						
Overall Qualit	y Detern	nination	High				

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any
	gross findings in related tissues
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9
	weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments
Domain 1: Reporting Quality				
Metr	ric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Perfo	ormance			
Metr	ric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).
Met	ric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.
Domain 3: Confounding / Vari	able Con	trol		
Met	ric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.
Domain 4: Selective Reporting and Attrition				
Continued on next page				

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to Sprague-Dawley rats in the diet: Final report.

TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered

Diethylhexyl Phthalate

Study Citation:

Health Outcome(s) Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched and Reported posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissues.-Ocular/Sensory-Clinical signs: Discharge Health Effect(s): from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues **Duration and** Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation **Exposure Route:** (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- postnatal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Species: Rat-Sprague-Dawley - [rat]-Both Chemical: Diethylhexyl Phthalate- Parent compound **HERO ID:** 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685 Domain Metric Comments Rating Selective Reporting and Attrition Metric 5: High Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1." Domain 5: Exposure Methods Sensitivity Metric 6: Chemical administration and High The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food characterization was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type. Metric 7: Exposure timing, frequency, and High The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development duration as groups of animals age. Domain 6: Outcome Measures and Results Display Metric 8: Endpoint sensitivity and specificity Low The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 16 of 21

			continued from p	revious page			
Study Citation:	TherImmun	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered					
Health Outcome(s) and Reported Health Effect(s): Duration and	InerImmune Research Corporation, (2004). Diethylnexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 davs)-F0- lactation						
Exposure Route: Species: Chemical: HERO ID:	(21 days)-F natal (weani (21 days)-F PND81))-F1 weeks cohat Rat-Sprague Diethylhexy 3108900 Lin	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	High				

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - premating (~60 days (PND21 to PND81))-F1 - mating (9 weeks cohabitation)-F1 - post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1 - post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1 - post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical: HERO ID:	Diethylhexyl Phthalate- Parent compound 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments		
Domain 1: Reporting Quality						
Met	tric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.		
Domain 2: Selection and Perfo	ormance					
Met	tric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).		
Met	tric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.		
Domain 3: Confounding / Variable Control						
Met	tric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.		
Domain 4: Selective Reporting and Attrition						
Continued on next page						

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

			-						
Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered								
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues- Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues- urroes findings in related tissues								
Duration and Exposure Poute:	Oral-Diet-Di	uration: Reproductive/Developmental-3-F	F0- premating	(6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (\sim 21 days)-F0- lactation					
Exposure Route.	natal (weaning	ng through necropsy)-F2- premating (~60	days (PND21	to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation					
	(21 days)-F2 PND81))-F1	 P- post-natal (weaning through necropsy) mating (9 weeks cohabitation)-F1- post 	-F0- prematin st-natal (wean	g (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to ing through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9					
Species:	weeks cohab	weeks cohabitation) F2- post-natal (weaning through necropsy) F2- premating (50 days (F1D21 to F1D01)) F2- mating (5 Pet Seregue Deviley, Fet Roth							
Chemical:	Diethylhexy	Diethylhexyl Phthalate- Parent compound							
HERO ID:	3108900 Lin	ked HERO ID(s): 3108900, 1334515, 55:	56685						
Domain	M 5		Rating	Comments					
	Metric 5:	Selective Reporting and Attrition	Hign	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."					
Domain 5: Exposure Me	thods Sensitiv	ity							
·	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.					
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.					
Domain 6: Outcome Measures and Results Display									
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17					

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mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 17 of 21

continued from previous page							
Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered						
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross finding						
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound 3108900 Linked HERO ID(s); 3108900, 1334515, 5556685						
Domain		Metric	Rating	Comments			
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				
Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered						
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	to Sprague-Dawley rats in the diet: Final report.						
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched						
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge						
Health Effect(s):	from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:,						
	urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy,						
	malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and						
	histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-						
	Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any						
	gross findings in related tissues						
Duration and	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation						
Exposure Route:	(21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-						
	natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation						
	(21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to						
	PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9						
	weeks cohabitation)-F2- post-natal (weaning through necropsy)						
Species:	Rat-Sprague-Dawley - [rat]-Both						
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685						

Domain		Metric	Rating	Comments		
Domain 1: Reporting Q	Domain 1: Reporting Quality					
	Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.		
Domain 2: Selection and	d Performance					
	Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups. Offspring of each generation were randomly selected for various outcomes (e.g., mating, necropsy, etc).		
	Metric 3:	Observational Bias / Blinding Changes	High	Blinding was not reported for the main study, but the outcomes of interest were not subjective nature, or blinding was not required. Additional histological analysis of some tissues was conducted by the pathology working group (PWG), and these examinations were conducted without prior knowledge of the dose groups.		
Domain 3: Confounding	g / Variable Co	ntrol				
	Metric 4:	Confounding / Variable Control	High	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A background level of 1.5 ppm was A positive control is not required for the study type. The control responses were appropriate. There was no consistent evidence of palatability issues or other confounding factors.		
Domain 4: Selective Reporting and Attrition						
	Continued on next page					

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Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

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Study Citation:	TherImmune	e Research Corporation, (2004). Diethylhex	xylphthalate:	Multigenerational reproductive assessment by continuous breeding when administered	
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross necropsy and histopa				
Duration and Exposure Route: Species: Chemical:	gross findings in related tissues Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post- natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- post-natal (weaning through necropsy) Rat-Sprague-Dawley - [rat]-Both				
HERO ID:	3108900 Lin	ked HERO ID(s): 3108900, 1334515, 555	6685		
Domain		Metric	Rating	Comments	
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."	
Domain 5: Exposure Me	ethods Sensitiv Metric 6:	ity Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.	
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breed- ing study and the outcomes of interest including additional assessments of development as groups of animals age.	
Domain 6: Outcomo Ma	aguras and Day	sulta Diaplay			
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.	

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 3108900 Table: 18 of 21

Diethylhexyl Phthalate	

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		previous	P"B"

Study Citation: Health Outcome(s)	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched				
and Reported Health Effect(s):	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Benavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOcular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-				
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F2- lactation (21 days)-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F2- premating (~60 days (PND21 to PND81))-F2- premating (~60 days (PND21 to PND81))-F2- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)				
Species:	Rat-Sprague-Dawley - [rat]-Both				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 55	56685			
Domain	Metric	Rating	Comments		
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.		
Additional Comments:	None				

Overall Quality Determination

High

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOther (please specify below) (General clinical
Health Effect(s):	signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia,
	dyspnea, missing anatomy, malocclusion, papilloma, obesity
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-
Exposure Route:	lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Qu	ality Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All ani-	
				mal husbandry details were reported including the number of animals per cage. Expo- sure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and	Performance				
	Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for the main study, but the outcomes of interest were not subjective in nature, or blinding was not required.	
Domain 3: Confounding	/ Variable Cor	ntrol			
	Metric 4:	Confounding / Variable Control	Medium	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A positive control is not required for the study type. The control responses were appropriate. There were no differences in food intake (g/animal/day) across groups prior to and throughout mating or gestation. Feed intake (g/kg BW) in high-dose males was significantly decreased in the absence of body weight changes. Food and water consumption in dams was decreased in a dose-related manner during lactation, but no differences in final body weights were observed. It is unclear if the decreases were related to palatability issues.	
Domain 4: Selective Ren	orting and Att	rition			
F	Metric 5:	Selective Reporting and Attrition	High	No adults died and data were reported for all specified outcomes. There was no indica- tion of issues with animal attrition or selective reporting.	
Domain 5: Exposure Methods Sensitivity					
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.	
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Diethylhexyl Phthalate

		conti	inued from previ	ous page
Study Citation: Health Outcome(s) and Reported Health Effect(s):	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOther (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dwamae, missing enterty			
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0- lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685			
Domain		Metric	Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome M	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	Animals were observed for clinical signs, and low incidences of clinical signs relevant to the skin, neurological, and other (general) outcomes were reported. No further eval- uation of these target organs/systems was conducted (e.g., histopathology) and clinical signs are not considered to be a sensitive measure of target organ toxicity. There are no concerns regarding sampling, and all dose groups were evaluated.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			

Overall Quality Determination

Medium

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOther (please specify below) (General clinical
Health Effect(s):	signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia,
	dyspnea, missing anatomy, malocclusion, papilloma, obesity
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-
Exposure Route:	lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments	
Domain 1: Reporting Qua	ality Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.	
Domain 2: Selection and	Performance				
	Metric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for the main study, but the outcomes of interest were not subjective in nature, or blinding was not required.	
Domain 3: Confounding	/ Variable Con	trol			
	Metric 4:	Confounding / Variable Control	Medium	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A positive control is not required for the study type. The control responses were appropriate. There were no differences in food intake (g/animal/day) across groups prior to and throughout mating or gestation. Feed intake (g/kg BW) in high-dose males was significantly decreased in the absence of body weight changes. Food and water consumption in dams was decreased in a dose-related manner during lactation, but no differences in final body weights were observed. It is unclear if the decreases were related to palatability issues.	
Domain 4: Selective Rend	orting and Att	rition			
	Metric 5:	Selective Reporting and Attrition	High	No adults died and data were reported for all specified outcomes. There was no indica- tion of issues with animal attrition or selective reporting.	
Domain 5: Exposure Methods Sensitivity					
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.	
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May 2025 Human Health Hazard Animal Toxicology Evaluation

Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported Health Effect(s):	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOther (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dwamae, missing enterty			
Duration and Exposure Route: Species: Chemical: HERO ID:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0- lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days) Rat-Sprague-Dawley - [rat]-Both Diethylhexyl Phthalate- Parent compound 3108900 Linked HERO ID(s): 3108900, 1334515, 5556685			
Domain		Metric	Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome M	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	Animals were observed for clinical signs, and low incidences of clinical signs relevant to the skin, neurological, and other (general) outcomes were reported. No further eval- uation of these target organs/systems was conducted (e.g., histopathology) and clinical signs are not considered to be a sensitive measure of target organ toxicity. There are no concerns regarding sampling, and all dose groups were evaluated.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.
Additional Comments:	None			

Overall Quality Determination

Medium

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered
	to Sprague-Dawley rats in the diet: Final report.
Health Outcome(s)	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched
and Reported	posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOther (please specify below) (General clinical
Health Effect(s):	signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia,
	dyspnea, missing anatomy, malocclusion, papilloma, obesity
Duration and	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (1 week)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-
Exposure Route:	lactation (21 days)-F0- premating (1 week)-F0- mating (cohabitation for 28 days)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain		Metric	Rating	Comments
Domain 1: Reporting Quality	ty			
N	Aetric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used CrI:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
Domain 2: Selection and Pe	erformance			
Ν	Aetric 2:	Allocation	High	Animals were randomly assigned to groups using a computer-generated randomization procedure that ensured equal weight distribution between groups.
N	Aetric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported for the main study, but the outcomes of interest were not subjective in nature, or blinding was not required.
Domain 3: Confounding / V	/ariable Con	trol		
W	Aetric 4:	Confounding / Variable Control	Medium	The study utilized a concurrent negative control group (animals fed normal diets not containing the test substance). A positive control is not required for the study type. The control responses were appropriate. There were no differences in food intake (g/animal/day) across groups prior to and throughout mating or gestation. Feed intake (g/kg BW) in high-dose males was significantly decreased in the absence of body weight changes. Food and water consumption in dams was decreased in a dose-related manner during lactation, but no differences in final body weights were observed. It is unclear if the decreases were related to palatability issues.
Domain 4: Selective Report	ting and Attr	ition		
N	Aetric 5:	Selective Reporting and Attrition	High	No adults died and data were reported for all specified outcomes. There was no indica- tion of issues with animal attrition or selective reporting.
Domain 5: Exposure Metho	ods Sensitivi	tv		
	Aetric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was pro- vided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
		Contin	nued on next pa	ge

May 2025

Diethylhexyl Phthalate

Human Health Hazard Animal Toxicology Evaluation

		conti	nued from previ	ous page	
Study Citation:	TherImmun	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered			
Health Outcome(s) and Reported Health Effect(s):	to Sprague-Dawley rats in the diet: Final report. Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissuesOther (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea_missing_anatomy_malocclusion_panilloma_obesity_				
Duration and	Oral-Diet-D	uration: Reproductive/Developmental-1-F0)- premating (1 w	eek)-F0- mating (cohabitation for 28 days)-F0 - gestation (time not specified)-F0-	
Exposure Route:	lactation (21	days)-F0- premating (1 week)-F0- mating	(cohabitation for	28 days)	
Species:	Rat-Sprague	e-Dawley - [rat]-Both			
Chemical:	Diethylhexy	l Phthalate- Parent compound			
HERO ID:	3108900 Lii	nked HERO ID(s): 3108900, 1334515, 5550	6685		
Domain		Metric	Rating	Comments	
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.	
Domain 6: Outcome Me	easures and Re	sults Display			
	Metric 8:	Endpoint sensitivity and specificity	Low	Animals were observed for clinical signs, and low incidences of clinical signs relevant to the skin, neurological, and other (general) outcomes were reported. No further eval- uation of these target organs/systems was conducted (e.g., histopathology) and clinical signs are not considered to be a sensitive measure of target organ toxicity. There are no concerns regarding sampling, and all dose groups were evaluated.	
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means \pm SE, and individual animal data were provided.	
Additional Comments:	None				
Overall Quali	ty Deteri	nination	Medium		

Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411.
Health Outcome(s)	Reproductive/Developmental-GD21 Sacrifice:Number of male fetuses, Average male fetus body weight, Androgen receptor expression in testes by im-
and Reported	munohistochemistry analysis, Serum testosterone and LH levels, Gene expression in testes (cDNA microarray and RT-PCR).PND 63 Sacrifice:Litter size,
Health Effect(s):	Offspring body weight (PND 1), Male-female ratio, Male offspring body weights (measured weekly from PND 1-63), Male offspring body weights (on
	PND 63), Male offspring clinical signs, Number of areolae/male offspring, Anogenital distance of male offspring, Organ weights of male offspring (testis,
	epididymis, prostate), Sperm concentration of male offspring, Sperm motility and viability of male offspring, Androgen receptor expression in testes by
	immunohistochemistry analysis, Serum testosterone and LH levels.
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21)
Exposure Route:	
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697710

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); expo- sure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal charac- teristics (starting body weight; parity not explicitly stated), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance			
Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
Metric 3:	Observational Bias / Blinding Changes	Medium	The study authors state that anogenital distances were measured blindly. Although blinding was not reported for other endpoints measured, most of the endpoints of interest were objective or simple measures. Sperm counts were conducted using a hemacytometer which may be subjective.
Domain 3: Confounding / Variable Con	ntrol		
Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
	Contin	nued on nex	t page

Human Health Hazard Animal Toxicology Evaluation

		ر continued from ا	previous page		
Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male				
Health Outcome(s) and Reported Health Effect(s):	reproductive/Developmental-GD21 Sacrifice:Number of male fetuses, Average male fetus body weight, Androgen receptor expression in testes by im- munohistochemistry analysis, Serum testosterone and LH levels, Gene expression in testes (cDNA microarray and RT–PCR).PND 63 Sacrifice:Litter size, Offspring body weight (PND 1), Male-female ratio, Male offspring body weights (measured weekly from PND 1-63), Male offspring body weights (on PND 63), Male offspring clinical signs, Number of areolae/male offspring, Anogenital distance of male offspring, Organ weights of male offspring (testis, epididymis, prostate), Sperm concentration of male offspring, Sperm motility and viability of male offspring, Androgen receptor expression in testes by immunohistochemistry analysis, Serum testosterone and LH levels.				
Exposure Route	Orai-Gavage-Duration. Reproductive/Deve	iopinentai-1-10 - gestati	(df) (df) 11-21)		
Species:	Rat-Sprague-Dawley - [rat]-Female				
Chemical:	Diethylhexyl Phthalate- Parent compound				
HERO ID:	697710				
Domain	Metric	Rating	Comments		
Domain 4: Selective Re	eporting and Attrition				
	Metric 5: Selective Reporting and Att	ition Low	Quantitative results were reported for most, but not all outcomes described in the meth- ods. It was stated that the number of male fetuses on GD 21 was recorded, however, no results were provided for this metric. For gene expression results from the cDNA mi- croarray, only results for the animals receiving 100 or 500 mg/kg bw/day are provided. The absence of the 10 mg/kg bw/day group is not explained in the text. In addition, the authors reference Table 4-d in the text, however, there is no Table 4-d included in the paper. This suggests that some data may be missing from the paper. The authors stated that offspring body weights and the male-female ratio per litter were recorded on PND		

			spring were weighed weekly between PND 1 and 63, however, these measured weights are not reported. Overall, these omissions are expected to significantly impact the in- terpretation of the results. Sample sizes were not clearly reported; there is insufficient information to determine whether any animal attrition occurred.
Domain 5: Exposure Methods Sensiti	vity		
Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Pure Chemical Industries), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed; however, a certificate of analysis was likely available from the supplier. The authors reported doses in mg/kg bw/day; test substance concentrations in the test solutions were not analytically measured. It is also unclear whether doses were adjusted for daily body weight changes. Storage conditions were not reported. The gavage volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage volumes of the treatment groups were the same. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
Metric 7:	Exposure timing, frequency, and	Medium	For this study, the route, frequency, and duration of exposure (dams exposed orally via

Exposure timing, frequency, and duration Medium For this study, the route, frequency, and duration of exposure (dams exposed orally via gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of GD 11 to 21 and did not include the period of organogenesis.

1, however, no results were provided for these metrics. In addition, it was stated that off-

Domain 6: Outcome Measures and Results Display

Human Health Hazard Animal Toxicology Evaluation

	continued from previous page
Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male
	reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411.
Health Outcome(s)	Reproductive/Developmental-GD21 Sacrifice:Number of male fetuses, Average male fetus body weight, Androgen receptor expression in testes by im-
and Reported	munohistochemistry analysis, Serum testosterone and LH levels, Gene expression in testes (cDNA microarray and RT-PCR).PND 63 Sacrifice:Litter size,
Health Effect(s):	Offspring body weight (PND 1), Male-female ratio, Male offspring body weights (measured weekly from PND 1-63), Male offspring body weights (on
	PND 63), Male offspring clinical signs, Number of areolae/male offspring, Anogenital distance of male offspring, Organ weights of male offspring (testis,
	epididymis, prostate), Sperm concentration of male offspring, Sperm motility and viability of male offspring, Androgen receptor expression in testes by

697710			
Diethylhexyl Phthalate- Parent compound			
Rat-Sprague-Dawley - [rat]-Female			
Oral-Gavage-Duration: Reproductive/Develop	pmental-1-F0 - gestation (GD 11-21)		
immunohistochemistry analysis, Serum testos	sterone and LH levels.		
epididyinis, prostate), Sperin concentration o	in male onspring, Sperm mounty and	viability of male offspring, Androgen receptor expression in testes t	Jy
anididumia mastata). Sname concentration of	of male offering. Snorm metility and	vishility of male offenning. Andreasen resenter every signing (test	
PND 63) Male offspring clinical signs Num	her of areolae/male offspring Anogen	ital distance of male offspring. Organ weights of male offspring (testi	is
Offspring body weight (PND 1), Male-femal	le ratio, Male offspring body weights	(measured weekly from PND 1-63), Male offspring body weights (c	on
munohistochemistry analysis, Serum testoster	rone and LH levels, Gene expression i	n testes (cDNA microarray and RT-PCR).PND 63 Sacrifice:Litter siz	æ,
Reproductive/Developmental-GD21 Sacrifice	e:Number of male fetuses, Average m	ale fetus body weight, Androgen receptor expression in testes by in	n-
reproductive organs in a rat model. Journal of	f Reproduction and Development 55(4	-):400-411.	
	reproductive organs in a rat model. Journal of Reproductive/Developmental-GD21 Sacrifice munohistochemistry analysis, Serum testoste Offspring body weight (PND 1), Male-femal PND 63), Male offspring clinical signs, Num epididymis, prostate), Sperm concentration of immunohistochemistry analysis, Serum testos Oral-Gavage-Duration: Reproductive/Develo Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound	reproductive organs in a rat model. Journal of Reproduction and Development 55(4 Reproductive/Developmental-GD21 Sacrifice:Number of male fetuses, Average m munohistochemistry analysis, Serum testosterone and LH levels, Gene expression i Offspring body weight (PND 1), Male-female ratio, Male offspring body weights PND 63), Male offspring clinical signs, Number of areolae/male offspring, Anogen epididymis, prostate), Sperm concentration of male offspring, Sperm motility and immunohistochemistry analysis, Serum testosterone and LH levels. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21) Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound	reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411. Reproductive/Developmental-GD21 Sacrifice:Number of male fetuses, Average male fetus body weight, Androgen receptor expression in testes by in munohistochemistry analysis, Serum testosterone and LH levels, Gene expression in testes (cDNA microarray and RT–PCR).PND 63 Sacrifice:Litter siz Offspring body weight (PND 1), Male-female ratio, Male offspring body weights (measured weekly from PND 1-63), Male offspring body weights (c PND 63), Male offspring clinical signs, Number of areolae/male offspring, Anogenital distance of male offspring, Organ weights of male offspring (testi epididymis, prostate), Sperm concentration of male offspring, Sperm motility and viability of male offspring, Androgen receptor expression in testes to immunohistochemistry analysis, Serum testosterone and LH levels. Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21) Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	This was a non-guideline in-utero developmental toxicity study. The test animals (rats) and sex (females) were appropriate for the evaluation of the endpoints. The study authors state that "the testes of four fetuses were collected and fixed in Bouin's solution for immunohistochemical analysis. The other testes were used for RNA isolation (pooled samples)". It is not entirely clear how many testes and from how many animals were used for immunohistochemical analysis versus RNA isolation. This uncertainty in the number of animals used for each analysis is expected to substantially impact the interpretation of the results. In addition, the authors state that fetuses were fixed in Bouin's solution for immunohistochemical analysis. Fixing testis with Bouin's solution is acceptable, but modified Davidson's solution is preferred. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose selection, and a NOAEL was not derived. The number of animals per group (8 dams/group) was small although this was a non-guideline study; no power analysis was conducted. The sample sizes for some endpoints were not reported in the interpretation of the study results. The methods state that n = 10 male offspring/group were separated from mothers on PND 22, but do not explicitly state whether all of these males were used for measuring downstream endpoints. Methods for most reproductive endpoints were adequately described and were appropriate and sensitive to the outcomes of interest. Methods for measuring sperm motility were cited to another publication.
	Metric 9:	Results presentation	Low	Quantitative data (mean \pm SD) were provided for most endpoints. Fold-changes (from pooled treated vs. control samples) were provided for gene expression analysis by cDNA microarray. Statistical significance was provided for these data and statistical methods were generally described. There is no indication that the litter was used as the statistical unit for fetal endpoints, and insufficient data were provided to conduct an independent statistical analysis. Qualitative data (representative images from immuno-histochemistry staining of testes) was provided for androgen receptor expression in the testes. Relative testis weights were provided in the absence of absolute weights. Absolute epididymis and prostate weights were also not reported. No results were provided for the number of male fetuses/litter, male offspring body weight (PND 1), male-female ratio, and male offspring body weights from PND 1-63. Sample sizes were not included in any data tables.

Additional Comments: None

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697710 Table: 1 of 4

	col	ntinued from previous	s page				
Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Ch reproductive organs in a rat model. Journal of Repro	oi, K., Jeung, E. (200 duction and Developm	9). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male ent 55(4):400-411.				
Health Outcome(s)	Reproductive/Developmental-GD21 Sacrifice:Numb	per of male fetuses, Av	erage male fetus body weight, Androgen receptor expression in testes by im-				
and Reported	munohistochemistry analysis, Serum testosterone an	d LH levels, Gene exp	ression in testes (cDNA microarray and RT-PCR).PND 63 Sacrifice:Litter size,				
Health Effect(s):	Offspring body weight (PND 1), Male-female ratio,	, Male offspring body	weights (measured weekly from PND 1-63), Male offspring body weights (on				
	PND 63), Male offspring clinical signs, Number of a	reolae/male offspring,	Anogenital distance of male offspring, Organ weights of male offspring (testis,				
	epididymis, prostate), Sperm concentration of male	offspring, Sperm moti	lity and viability of male offspring, Androgen receptor expression in testes by				
	immunohistochemistry analysis, Serum testosterone and LH levels.						
Duration and	Oral-Gavage-Duration: Reproductive/Developmenta	ll-1-F0 - gestation (GD	11-21)				
Exposure Route:							
Species:	Rat-Sprague-Dawley - [rat]-Female						
Chemical:	Diethylhexyl Phthalate- Parent compound						
HERO ID:	697710						
Domain	Metric	Rating	Comments				
Overall Quali	ity Determination	Low					

Domain 3: Confounding / Variable Control

Domain 4: Selective Reporting and Attrition

Domain 5: Exposure Methods Sensitivity

Metric 4:

Metric 5:

Confounding / Variable Control

Selective Reporting and Attrition

The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to significantly impact

No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors. This lack of data provided for these endpoints resulted in an uninforma-

these endpoints (maternal body weights and clinical signs).

tive designation.

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411. Nutritional/Metabolic-Maternal body weight-Other (please specify below) (Clinical signs)-Observed maternal clinical signs-Neurological/Behavioral-Observed maternal abnormal behavior Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21) Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697710 			
Domain		Metric	Rating	Comments
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Low	All critical and some important information were reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); expo- sure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal char- acteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test sub- stance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection an	d Performance			
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, the endpoints are simple measures (body weights). Blinding was not reported for clinical signs.

Medium

Uninformative

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Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697710 Table: 2 of 4

		continued from previous page				
Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., reproductive organs in a rat model. Journal of Rep	Choi, K., Jeung, E. (2009). Differential efferorduction and Development 55(4):400-411.	ects of flutamide and di-(2-ethylhexyl) phthalate on male			
Health Outcome(s)	Nutritional/Metabolic-Maternal body weight-Oth	her (please specify below) (Clinical signs)-O	bserved maternal clinical signs-Neurological/Behavioral-			
and Reported	Observed maternal abnormal behavior					
Health Effect(s):						
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21)					
Exposure Route:						
Species:	Rat-Sprague-Dawley - [rat]-Female					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	697710					
Domain	Metric	Rating	Comments			

Domain		Metric	Kaung	Comments
	Metric 6:	Chemical administration and	Low	In this study, test animals were gavaged with DEHP. The source of the test substance
		characterization		was reported (Wako Pure Chemical Industries), however, the purity of the test substance
				was not provided. In addition, independent analytical verification of the test substance
				not performed, nowever, a certificate of analysis was fixely available from the sup-
				test solutions were not analytically measured. It is also unclear whether doses were ad-
				justed for daily body weight changes. Storage conditions were not reported. The gavage
				volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage
				volumes of the treatment groups were the same. There were very few details provided
				on test substance preparation for gavage. These uncertainties in the exposure characteri- zation are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and	High	For this study, the route, frequency, and duration of exposure (dams exposed orally via
		duration		gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However,
				the study authors did not provide an explanation for why they selected an exposure
Domain 6: Outcome Me	easures and Re	esults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an in-utero developmental toxicity study. The study does not state whether
				clinical signs were cage-side or detailed clinical observations with handling. The fre-
				quency of observations was not specified. The authors also do not give any information
				on the "abnormal behavior" observations. The number of exposure groups (0, 10, 100,
				and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose
				the endpoints of interest
	Metric 9.	Results presentation	Uninformative	No results were provided for maternal body weights observed clinical signs or abnor-
				mal behaviors.
Additional Comments:	None			
Overall Qualif	ty Deter	mination	Uninformative	
	y Duur			

gavage. A positive control group receiving testosterone propionate, an androgen agonist,

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Species: Chemical: HERO ID:	 Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411. Nutritional/Metabolic-Maternal body weight-Other (please specify below) (Clinical signs)-Observed maternal clinical signs-Neurological/Behavioral-Observed maternal abnormal behavior Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21) Rat-Sprague-Dawley - [rat]-Female Diethylhexyl Phthalate- Parent compound 697710 				
Domain		Metric	Rating	Comments	
Domain 1: Reporting Q	uality Metric 1:	Reporting Quality	Low	All critical and some important information were reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); expo- sure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal char- acteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test sub- stance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.	
Domain 2: Selection and	d Performance		-		
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.	
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, the endpoints are simple measures (body weights). Blinding was not reported for clinical signs.	
Domain 3: Confounding	g / Variable Con Metric 4:	ntrol Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by	

		Continued on next page .	••
Domain 5: Exposure Methods Sensitiv	ity		
Domain 4: Selective Reporting and Att Metric 5:	rition Selective Reporting and Attrition	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnor- mal behaviors. This lack of data provided for these endpoints resulted in an uninforma- tive designation.
			by gavage, was used as an indicator of androgenic activity. Animal husbandry condi- tions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and wa- ter dispensing containers were not described. This is not expected to significantly impact these endpoints (maternal body weights and clinical signs).

Human Health Hazard Animal Toxicology Evaluation

HERO ID: 697710 Table: 3 of 4

		continued from previous page				
Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., reproductive organs in a rat model. Journal of Ref	Choi, K., Jeung, E. (2009). Differential effe production and Development 55(4):400-411.	ects of flutamide and di-(2-ethylhexyl) phthalate on male			
Health Outcome(s)	Nutritional/Metabolic-Maternal body weight-Oth	er (please specify below) (Clinical signs)-O	bserved maternal clinical signs-Neurological/Behavioral-			
and Reported	Observed maternal abnormal behavior					
Health Effect(s):						
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21)					
Exposure Route:						
Species:	Rat-Sprague-Dawley - [rat]-Female					
Chemical:	Diethylhexyl Phthalate- Parent compound					
HERO ID:	697710					
Domain	Metric	Rating	Comments			

Domain		Metric	Kaung	Comments
	Metric 6:	Chemical administration and	Low	In this study, test animals were gavaged with DEHP. The source of the test substance
		characterization		was reported (Wako Pure Chemical Industries), however, the purity of the test substance
				was not provided. In addition, independent analytical verification of the test substance
				not performed, nowever, a certificate of analysis was fixely available from the sup-
				test solutions were not analytically measured. It is also unclear whether doses were ad-
				justed for daily body weight changes. Storage conditions were not reported. The gavage
				volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage
				volumes of the treatment groups were the same. There were very few details provided
				on test substance preparation for gavage. These uncertainties in the exposure characteri- zation are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and	High	For this study, the route, frequency, and duration of exposure (dams exposed orally via
		duration		gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However,
				the study authors did not provide an explanation for why they selected an exposure
Domain 6: Outcome Me	easures and Re	esults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an in-utero developmental toxicity study. The study does not state whether
				clinical signs were cage-side or detailed clinical observations with handling. The fre-
				quency of observations was not specified. The authors also do not give any information
				on the "abnormal behavior" observations. The number of exposure groups (0, 10, 100,
				and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose
				the endpoints of interest
	Metric 9.	Results presentation	Uninformative	No results were provided for maternal body weights observed clinical signs or abnor-
				mal behaviors.
Additional Comments:	None			
Overall Qualif	ty Deter	mination	Uninformative	
	y Duur			

gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry condi-

Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Spacing:	Vo, T., Jung reproductive Nutritional/I Observed ma Oral-Gavage	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411. Nutritional/Metabolic-Maternal body weight-Other (please specify below) (Clinical signs)-Observed maternal clinical signs-Neurological/Behavioral- Observed maternal abnormal behavior Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21)					
Chemical:	Diethylhexy	Phthalate- Parent compound					
HERO ID:	697710						
Domain		Metric	Rating	Comments			
Domain 1: Reporting Q	uality		-				
	Metric 1:	Reporting Quality	Low	All critical and some important information were reported in this study. The study in- cluded identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); expo- sure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal char- acteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test sub- stance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.			
Domain 2: Selection an	d Performance						
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.			
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, the endpoints are simple measures (body weights). Blinding was not reported for clinical signs.			
Domain 3: Confounding	g / Variable Co	ntrol					
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by			

tions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to significantly impact these endpoints (maternal body weights and clinical signs). Domain 4: Selective Reporting and Attrition Metric 5: Selective Reporting and Attrition Uninformative No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors. This lack of data provided for these endpoints resulted in an uninformative designation.

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HERO ID: 697710 Table: 4 of 4

	continued from previous page
Study Citation:	Vo, T., Jung, E., Dang, V., Jung, K., Baek, J., Choi, K., Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. Journal of Reproduction and Development 55(4):400-411.
Health Outcome(s)	Nutritional/Metabolic-Maternal body weight-Other (please specify below) (Clinical signs)-Observed maternal clinical signs-Neurological/Behavioral-
and Reported	Observed maternal abnormal behavior
Health Effect(s):	
Duration and	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 11-21)
Exposure Route:	

Species:Rat-Sprague-Dawley - [rat]-FemaleChemical:Diethylhexyl Phthalate- Parent compoundHERO ID:697710

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Pure Chemical Industries), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed; however, a certificate of analysis was likely available from the supplier. The authors reported doses in mg/kg bw/day; test substance concentrations in the test solutions were not analytically measured. It is also unclear whether doses were adjusted for daily body weight changes. Storage conditions were not reported. The gavage volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage volumes of the treatment groups were the same. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (dams exposed orally via gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of GD 11 to 21.
Domain 6: Outcome Me	easures and Re	sults Display		
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an in-utero developmental toxicity study. The study does not state whether clinical signs were cage-side or detailed clinical observations with handling. The fre- quency of observations was not specified. The authors also do not give any information on the "abnormal behavior" observations. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose selection. The sample size (8 dams/group) was small but should have been sufficient for the endpoints of interest.
	Metric 9:	Results presentation	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnor- mal behaviors.
Additional Comments:	None			
Overall Qualit	ty Deteri	nination	Uninformative	

Study Citation: Health Outcome(s) and Reported	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221. Reproductive/Developmental-F0-treated dams (serum estradiol); F1 (oocyte meiosis and folliculogenesis, gene expression, DNA Methylation, protein levels of Stra8); F2 (folliculogenesis)							
Health Effect(s):								
Duration and	Oral-Gavage	e-Duration: Reproductive/Developmental-F	0 - gestation	(0.5-18.5 Days postcoitum)				
Exposure Route:								
Species:	Mouse-CD-1	l - [mouse]-Female						
Chemical:	Diethylhexy	I Phthalate- Parent compound						
	2319000		D. (*					
Domain Domain 1: Reporting Or	ality	Metric	Rating	Comments				
	Metric 1:	Reporting Quality	Low	All critical information was reported including the name of the test material (DEHP), the test species (mouse), the doses 0 and 40 ug/kg-day), duration of exposure (GD 0.5 to 18.5), and route (gavage), and quantitative results for at least one endpoint of information. Important information reported included the test species strain (CD-1), source (Vital River, Beijing, China), sex (females), animal husbandry conditions (temperature and light conditions), and outcome/endpoint evaluation methods. The study did not report test substance purity, animal age, starting body weights, parity, humidity, diet and water availability, number of per group, or number of animals per cage. The missing information is expected to significantly reduce the ability to evaluate the study.				
Domain 2: Selection and	l Performance Metric 2:	Allocation	Low	The method of allocation of animals into study groups, or downstream selection of ani- mals for various outcome assessments was not specified. There is no indication that ran- domization or other methods to control for important modifying factors across groups was used.				
	Metric 3:	Observational Bias / Blinding Changes	Low	The study did not report the use of blinding, but most of the outcomes of interest were not subjective in nature. One outcome, the assignment of follicular stages was likely subjective in nature, and blinding would have been appropriate.				
Domain 3: Confounding	y variable Coi Metric 4:	ntron Confounding / Variable Control	Medium	A concurrent negative vehicle control group was administered saline containing DMSO at a final concentration of 0.1%. The negative control responses appeared to be appropriate. The study did not evaluate standard potential variables of concern (e.g., food and water intake, body weights), and only minimal animal husbandry conditions were provided. DEHP is also a known endocrine disruptor and the outcomes of interest in this study were looking at effects related to endocrine disruption. It was not specified whether co-exposure to plasticizers was specifically avoided in this study or was at least consistent across groups. Positive controls were not included but were not necessary for this study.				
Domain 4: Selective Rep	porting and At	trition						
		Contin	nued on nex	ct page				

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Diethylhexyl Phthalate

		con	tinued from p	orevious page				
Study Citation:	Zhang, X. F induced by	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221.						
Health Outcome(s)	Reproductiv	Reproductive/Developmental-F0-treated dams (serum estradiol); F1 (oocyte meiosis and folliculogenesis, gene expression, DNA Methylation, protein						
and Reported	levels of Str	a8); F2 (folliculogenesis)						
Health Effect(s):								
Duration and	Oral-Gavage	e-Duration: Reproductive/Developmental	-F0 - gestation	n (0.5-18.5 Days postcoitum)				
Exposure Route:								
Species:	Mouse-CD-	1 - [mouse]-Female						
Chemical:	Diethylhexy	Phthalate- Parent compound						
HERO ID:	2519060							
Domain		Metric	Rating	Comments				
	Metric 5:	Selective Reporting and Attrition	Low	Selective reporting and attrition cannot be properly assessed because the study did not report the number of animals per group (one figure sampled 5 control and treated F0 dams, but it is not clear if this represents the number of animals per group). For most outcomes, it was only noted that most experiments were performed in triplicate (three independent trials), and some also included three replicates, but the actual sample sizes were also not specified. Data for all of the specified outcomes were reported.				
Domain 5: Exposure M	Iethods Sensitiv Metric 6:	vity Chemical administration and characterization	Low	The test substance was identified as diethylhexyl phthalate (DEHP) sourced from Sigma-Aldrich. The test material was not independently verified and the specific product (from Sigma) used in this study cannot be determined. The purity and/or grade were not reported and cannot be definitively verified on the supplier's website. The doses were not analytically verified. The method of exposure is assumed to be gavage; the study states that "water containing DEHP was given orally." In another study by the same group that appears to be on the same group of animals, it was indicated that the test substance was oraly delivered using an Eppendorf pipette with a disposable tip (Li et al., 2014; HERO 2519022). No gavage volume was specified and no details on the preparation (e.g., homogeneity, frequency), and stability of the test solutions were provided.				
	Metric 7:	Exposure timing, frequency, and duration	High	The dose, 40 ug/kg-day was justified by the study authors. The exposure timing, frequency, and duration were based on results from previous stud- ies. The exposure period (GD 0.5 to 18.5) covers the period of mammalian folliculoge- nesis was appropriate for the purpose of the study. The time of day of dosing was not specified. There is no indication that there were any inconsistencies across groups.				

Domain 6: Outcome Measures and Results Display

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Diethylhexyl Phthalate

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Study Citation: Health Outcome(s) and Reported Health Effect(s): Duration and Exposure Route: Spacies:	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221. Reproductive/Developmental-F0-treated dams (serum estradiol); F1 (oocyte meiosis and folliculogenesis, gene expression, DNA Methylation, protein levels of Stra8); F2 (folliculogenesis) Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (0.5-18.5 Days postcoitum)				
Chemical.	Diethylbey	1 - [mouse]-remain			
HERO ID:	2519060	1 i initiatate- i arent compound			
Domain	2317000	Metric	Rating	Comments	
	Metric 8:	Endpoint sensitivity and specificity	Low	The purpose of the study was to investigate whether maternal exposure to DEHP af- fected fetal ovarian development. The endpoints (measurement of serum estradiol on GD 12.5, gene expression and bisulfate sequencing on germ cells at GD 13.5, determi- nation of the meiotic status of oocytes on GD 17.5 in dames, and examination of ovaries from PND21 F1 and F2 offspring), were sensitive and appropriate for the purposes of the study. The details of the outcome assessment protocols were confusing and did not always clearly distinguish whether it was fetal or maternal samples being examined. The only figure reporting a sample size (n =5) was Fig. 2a, measuring estradiol levels in F0 pregnant dams. It was noted that some endpoints were assessed in triplicate. The outcomes were consistently assessed in the control and treatment groups. Only a single exposure group was tested, but the dose was justified by the study author, and the pur- pose of the study was not to identify a dose response. The test animal species/strain had been previously used by the same group and was obtained from a commercial source. It is unclear if the number of animals per group was appropriate because this information was not provided.	
	Metric 9:	Results presentation	Low	Data for all endpoints were reported primarily as figures showing means \pm SEM. Some figure legends specified that experiments were repeated at least three times, but did not specify how many animals were used to generate the data. Statistical significance was shown, and the methods of statistical analysis were adequately described.	

Additional Comments: None

Overall Quality Determination

Low