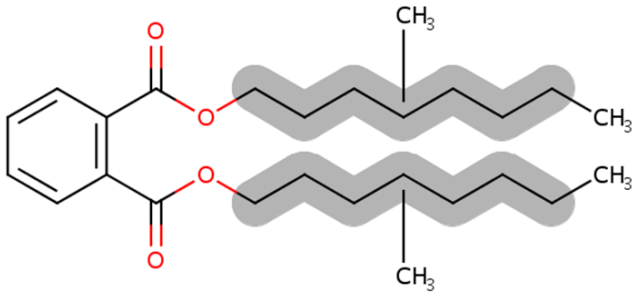
United States
Environmental Protection Agency

January 2025
Office of Chemical Safety and
Pollution Prevention

Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Diisononyl Phthalate (DINP)

Systematic Review Support Document for the Risk Evaluation

CASRNs: 28553-12-0 and 68515-48-0



January 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 *Risk Evaluation for Diisononyl Phthalate (DINP) - 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'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Risk Evaluation for Diisononyl Phthalate (DINP) - Systematic Review Protocol*.

Table of Contents

HERO ID	Reference	Page
Diisononyl Phthalate		
Short-term (>1-30 days)		
1325511	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.	6
11784564	Chen, J., Yang, S., Ma, B. C., Wang, J. L., Chen, J. X. (2022). Di-isononyl phthalate induces apoptosis and autophagy of mouse ovarian granulosa cells via oxidative stress. Ecotoxicology and Environmental Safety 242:113898.	14
7978479	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.	17
7978481	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.	20
11151638	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.	24
7978425	Chiu, K., Bashir, S. T., Nowak, R. A., Mei, W., Flaws, J. A. (2020). Subacute exposure to di-isononyl phthalate alters the morphology, endocrine function, and immune system in the colon of adult female mice. Scientific Reports 10(1):18788-18788.	30
1325350	Clewell, R. A., Sochaski, M., Edwards, K., Creasy, D. M., Willson, G., Andersen, M. E. (2013). Disposition of diiosononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats. Reproductive Toxicology 35(1):56–69.	32
697382	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.	34
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.	43
7978423	Liang, F., Yan, B. (2020). Oxidative damage in the liver and kidney induced by dermal exposure to diisononyl phthalate in Balb/c mice. Toxicology and Industrial Health 36(1):30-40.	47
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.	49
Subchronic (>30-91 days)		
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.	52
Chronic (>91 days)		
679889	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.	54

1325481 Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105]. **61**

680087 Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover. **69**

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. **76**

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. **82**

Reproductive/Developmental

11784571 Bhurke, A., Davila, J., Flaws, J. A., Bagchi, M. K., Bagchi, I. C. (2023). Exposure to di-isononyl phthalate during early pregnancy disrupts decidual angiogenesis and placental development in mice. Reproductive Toxicology 120:108446. **85**

1987588 Biomedical,, Exxon (1996). Reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) (sanitized). **87**

1987589 Biomedical,, Exxon (1996). Two generation reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) [unpublished] (sanitized). **90**

806135 Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209. **94**

1325348 Clewell, R. A., Thomas, A., Willson, G., Creasy, D. M., Andersen, M. E. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reproductive Toxicology 35(Elsevier):70-80. **101**

2510906 Furr, J. R., Lambright, C. S., Wilson, V. S., Foster, P. M., Gray, L. E., Jr (2014). A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. Toxicological Sciences 140(2):403-424. **107**

788239 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. **109**

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. **117**

11785000 Jr, Gray, L. E., Lambright, C. S., Evans, N., Ford, J., Conley, J. M. (2024). Using targeted fetal rat testis genomic and endocrine alterations to predict the effects of a phthalate mixture on the male reproductive tract. Current Research in Toxicology 7:100180. **125**

2807612 Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474. **128**

192872 Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170. **135**

680201 Waterman, S. J., Ambroso, J. L., Keller, L. H., Trimmer, G. W., Nikiforov, A. I., Harris, S. B. (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reproductive Toxicology 13(2):131-136. **142**

Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0

Chronic (>91 days)

1065989 Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386. **146**

Reproductive/Developmental

788239 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. **160**

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. **168**

Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 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 / Variable Control				
	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 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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Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547

Domain	Metric		Rating	Comments
	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 Methods Sensitivity				
	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 interest. 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was provided. The outcome methodology addressed the intended outcomes of interest and assessed 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 Quality Determination Medium

Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 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 / Variable Control				
	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 Reporting and Attrition				
	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 Methods Sensitivity				

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Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 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 interest. 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was provided. The outcome methodology addressed the intended outcomes of interest and assessed consistently across the study groups. The number of animals/group was appropriate (n=5/sex/group).
	Metric 9:	Results presentation	Uninformative	No information was provided on clinical signs.

Additional Comments: None

Overall Quality Determination Uninformative

Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
	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 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 / Variable Control			
	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 Reporting and Attrition			
	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.

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Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	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 interest. 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was provided. The outcome methodology addressed the intended outcomes of interest and assessed 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:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 1325547

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 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 / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	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”. Husbandry conditions were reported and similar. Significant difference in food intake was seen in females compared to control only at the very beginning of the study, and is thus unlikely to have an affect on the outcome . Water intake was not reported.
Domain 4: Selective Reporting and Attrition				
	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 Methods Sensitivity				

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Study Citation:	BIBRA, (1986). Rat liver and lipid effects of representative phthalate esters with EPA acknowlegement letter.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-24-21-day(s)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325511 Linked HERO ID(s): 1325511, 674933, 1325463, 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 interest. 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal studied was appropriate and justification for age and strain was provided. The outcome methodology addressed the intended outcomes of interest and assessed 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 High

Study Citation:	Chen, J., Yang, S., Ma, B. C., Wang, J. L., Chen, J. X. (2022). Di-isononyl phthalate induces apoptosis and autophagy of mouse ovarian granulosa cells via oxidative stress. Ecotoxicology and Environmental Safety 242:113898.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum estradiol, ovary histology, ovarian oxidative stress (levels of glutathione [GSH] and malondialdehyde [MDA], and activities of GSH-peroxidase [GSH-PX] and superoxide dismutase [SOD]), and apoptosis and autophagy-related protein levels in ovary via Western blot.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Mouse-Other (Kunming)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784564

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The chemical name, source, purity, and method of administration were all reported. The test animal species, source, strain, age, sex, and starting body weights were all reported. All animal husbandry details were reported to be standard, but no details were provided. However, all information on the exposure methods, experimental design, and endpoint evaluation methods were sufficiently described. 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	Low	This study is considered Low for Metric 2.1. 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	This study is considered Medium for Metric 2.2. Methods for histological evaluation were cited in previous paper (Wang et al. 2019) and implies ovaries were assessed blindly. The study did not report whether a secondary histopathology review was conducted. Blinding or other measures to reduce observational bias were not described for all other endpoints; however, the endpoints evaluated were not subjective in nature (e.g. serum estradiol, protein levels). The preponderance of objective outcomes for this target organ/system warrants a ranking of Medium for this metric.
Domain 3: Confounding / Variable Control				

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Study Citation:	Chen, J., Yang, S., Ma, B. C., Wang, J. L., Chen, J. X. (2022). Di-isononyl phthalate induces apoptosis and autophagy of mouse ovarian granulosa cells via oxidative stress. Ecotoxicology and Environmental Safety 242:113898.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum estradiol, ovary histology, ovarian oxidative stress (levels of glutathione [GSH] and malondialdehyde [MDA], and activities of GSH-peroxidase [GSH-PX] and superoxide dismutase [SOD]), and apoptosis and autophagy-related protein levels in ovary via Western blot.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Mouse-Other (Kunming)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784564

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	Low	This study is considered Low for Metric 3. A negative control was included however it is unclear if it was appropriate. The study authors do not report the vehicle the test substance was administered in or report if the control group received this vehicle. The biological response of the negative control was adequate. A positive control is not required for this study type. Husbandry conditions were not reported (referred to as standard conditions). The study did not report taking measures to minimize the exposure to other plasticizers. The type of cage animals were housed in or food and water dispensing containers were not reported. 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 weight changes were not reported, but the impact on results is expected to be minimal. The study measured estradiol level, which is influenced by the estrous stage. The study authors did not ensure all animals were dosed/sacrificed at the same point in their cycle.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	This study is considered Medium for Metric 4. Data were reported for all outcomes. The study authors do not report how many animals were treated/group, but do report in the figure legend data were obtained from six animals (n=6). It is unclear if there may have been animals that were treated and not included in the analysis. 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 that could influence the outcome assessment.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The vehicle the test substance was administered in was not reported. No details are provided on preparation, storage of stability of the test substance. Doses were not analytically verified. Gavage volume was not reported. It is unclear if volume was adjusted daily based on body weight. Source and vendor-reported purity were presented, but not independently verified. Details of exposure administration were insufficiently reported, and the missing information is likely to have a substantial impact on results.
	Metric 7:	Exposure timing, frequency, and duration	Low	This study is considered Low for Metric 5.2. The gavage dosing frequency (daily) and duration (14 days) is considered adequate. The study authors did not ensure all animals were dosed/sacrificed at point in their estrous cycle.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Chen, J., Yang, S., Ma, B. C., Wang, J. L., Chen, J. X. (2022). Di-isononyl phthalate induces apoptosis and autophagy of mouse ovarian granulosa cells via oxidative stress. Ecotoxicology and Environmental Safety 242:113898.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum estradiol, ovary histology, ovarian oxidative stress (levels of glutathione [GSH] and malondialdehyde [MDA], and activities of GSH-peroxidase [GSH-PX] and superoxide dismutase [SOD]), and apoptosis and autophagy-related protein levels in ovary via Western blot.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Mouse-Other (Kunming)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784564

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	High	This study is considered High for Metric 6.1. The species was appropriate to evaluate outcomes of interest. The study included three dose groups (2, 20, and 200 mg/kg/day) plus a control; the spacing was adequate to identify an apical NOAEL and LOAEL and to observe a dose-response relationship in reproductive outcomes. The methods of outcome assessment (histology, western blot, ELISA) were appropriate to evaluate mechanistic effects of ovarian toxicity and were consistently assessed across groups. Method to evaluated histology were cited in a published paper by the same laboratory (Wang et al. 2019). The number of animals per study group was appropriate (6/group).
	Metric 9:	Results presentation	Low	This study is considered Low for Metric 6.2. Data were analyzed and presented appropriately for effects on the ovary and the number of animals was provided. Quantitative data (means and standard deviations) were provided for serum estradiol, ovarian protein levels, and oxidative stress measurement. Statistical significance is presented in the figure. However, histological changes in the ovary were not adequately reported. Histological findings are briefly reported qualitatively without incidence data, severity scores, or reporting dose level significant effects were seen.

Additional Comments: An unspecified number of additional female mice were utilized for in vitro experiments to investigate the mechanisms of induction of apoptosis and autophagy in DINP-exposed ovarian granulosa cells. These mice were not exposed in vivo to DINP, and the in vitro experiments were not evaluated for data quality.

Overall Quality Determination Low

Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978479

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 μg/kg/day, 200 μg/kg/day, 20 mg/kg/day, and 200 mg/kg/day); DINP (20 μg/kg/day, 100 μg/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. 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 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	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 experimental/technical controls for sex hormone levels in sera are considered sufficient for proper analysis.
Domain 3: Confounding / Variable Control				

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Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978479

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	Medium	The study has minor confounds that may have minimally affected the results. For example, there is no indication of using randomization when assigning mice to their experimental group. Also, the chemical being used is not listed with all the relevant information 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 Reporting and Attrition				
	Metric 5:	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 Methods Sensitivity				
	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 μg/kg/day, 200 μg/kg/day, 20 mg/kg/day, and 200 mg/kg/day) and DINP 20 μg/kg/day, 100 μg/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 Measures and Results Display				

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Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl 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 analysis 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 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.
	Metric 9:	Results presentation	Medium	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 test) date in the study.

Additional Comments: None

Overall Quality Determination Medium

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) and Reported Health Effect(s):	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.
Duration and Exposure Route:	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)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978481

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 (DINP) 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 μg/kg/day, 100 μg/kg/day,20 mg/kg/day, 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 and 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 histological ID with no relation to treatment group. Other metrics did not state similar blinding, however, the objectivity (number of pups born) or experimental/technical controls for sex hormone levels in sera are considered sufficient for proper analysis.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study has minor confounds that may have minimally affected the results. For example, there is no indication of using randomization when assigning mice to their experimental group. Also, the chemical being used is not listed with all the relevant information 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.

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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) and Reported Health Effect(s):	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.
Duration and Exposure Route:	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)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978481

Domain	Metric	Rating	Comments
Domain 4: Selective Reporting and Attrition			
	Metric 5: 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: Exposure Methods Sensitivity			
	Metric 6: Chemical administration and characterization	Low	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. 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 μg/kg/day, 100 μg/kg/day, 20 mg/kg/day, 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			

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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) and Reported Health Effect(s):	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.
Duration and Exposure Route:	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)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	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 analysis of the reproductive/developmental toxicological effects of 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 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 measurements 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 development 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 average pup weight for the 12-month post-dosing group for 200 mg/kg/day DINP (n = 2 mice) and the percent female pups from the 12-month post-dosing group for 100 mg/kg/day DINP (n = 2 mice) and for 200 mg/kg/day (n = 1 mouse), was insufficient to perform statistical analysis. Some of the other groups for DINP 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 Analysis Core. Appropriate controls and calibrations were discussed and a sufficient sample size was reported for most groups except for the 15-month post-exposure group, which had sample sizes of 4 for some groups, which is low.

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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) and Reported Health Effect(s):	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.
Duration and Exposure Route:	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)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978481

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	The data within the study were presented in an accurate and transparent manner. Although 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 analyzed via ANOVA and a 2-sided Dunnett's pot hoc test). Also, there is no clear indication 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 authors 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 figure nor within the results section.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum estradiol levels
Duration and Exposure Route:	Oral-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11151638

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information were reported. The test material was identified as di-isononyl phthalate (DINP) (CAS no. 2855-3-12-0) and the source was reported. Other reported information included details on the test model (species, strain, source, and age); animal husbandry (food and water availability, temperature, humidity, light cycle); exposure details, experimental design, number of animals per group, endpoint evaluation methods, and results for the endpoint of interest. Missing information included the test chemical purity, number of animals/ per cage, and initial body weights.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors did not report how 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 evaluated were not subjective and used standard kits (e.g., ELISA).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. No positive control was included nor required for the study. Based on the data provided, study groups were maintained, exposed, and evaluated under comparable conditions. However, the consistency of other potentially confounding factors (body weights, food or water intake) was not reported. The study did not report taking measures to minimize the exposure to other plasticizers. Food, tap water, and bedding were not tested for contaminates, and the materials used to dispense water to the animals were not specified. The type of cage animals were housed in was not reported. It is unclear whether the presence of other endocrine disruptor contaminates would impact on the study results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in the results. Quantitative outcomes were reported for each group for most outcomes (colon weight not reported). The number of animals used for determining cytokine levels was 4-6/group; study authors did not provide an explanation. This is not expected to significantly impact results.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum estradiol levels
Duration and Exposure Route:	Oral-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11151638

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma-Aldrich) 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 at the time of purchase. There is no indication that the test substance was verified by the performing laboratory. Details on the preparation are provided in the cited reference (Chiu et al. 2020). No information was provided on the method used for mixing or how far in advance solutions were made. Storage or stability of the test solutions were not provided. Animals were dosed by gently pipetting chemical into the mouth (volume not reported). Dosing occurred at the same time each day. Doses were reported in mg/kg-day. It was not specified whether the doses were adjusted daily based on body weight, however, the exposure period was only 2 weeks. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The estrous cycle was monitored, and animals were sacrificed when they were in diestrus. This is appropriate since the authors were looking at estradiol levels; however, it also meant animals were not all exposed the same number of days. Exposure durations varied from 10-14 days- the exact duration within and across groups was not reported).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species and sample size are appropriate to evaluate outcomes of interest. A wide range of doses were studied (0.02 – 200 mg/kg/day). Doses were chosen to both mimic environmentally relevant exposure in humans (0.02 and 0.2 mg/kg/day) and to understand toxicological dose responses (2, 20, and 200 mg/kg/day). Outcomes were assessed consistently across study groups. There are no major concerns regarding the outcome methodology for outcomes of interest.
	Metric 9:	Results presentation	High	Data were fully reported as means +/- SEM. Statistical analysis was reported and appropriate. Statistical outliers in the estradiol assay were "identified and removed from analysis via ROUT."

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.
Health Outcome(s) and Reported Health Effect(s):	Immune/Hematological-Cytokine levels in colon via array (40 different cytokines) and ELISA (IL-1RA and CXCL12). Immunohistochemistry for goblet cells (MUC2). RNA levels of colon mRNA levels of immune and immune-related factors including: mucins (Muc1, 2, 3a, and 4), toll-like receptors (Trl4and 5), and specialized epithelial cells (ChgA [mature enteroendocrine cells], Lgr5 [stem cells], Lyz1 [Paneth cell marker], Cd24a [epithelial cell marker for cells located at bottom of intestinal crypts], and Vil1 [epithelium border]).
Duration and Exposure Route:	Oral-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11151638

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information were reported. The test material was identified as di-isononyl phthalate (DINP) (CAS no. 2855-3-12-0) and the source was reported. Other reported information included details on the test model (species, strain, source, and age); animal husbandry (food and water availability, temperature, humidity, light cycle); exposure details, experimental design, number of animals per group, endpoint evaluation methods, and results for the endpoint of interest. Missing information included the test chemical purity, number of animals/ per cage, and initial body weights.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors did not report how 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 evaluated were not subjective and used standard kits (e.g., ELISA).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. No positive control was included nor required for the study. Based on the data provided, study groups were maintained, exposed, and evaluated under comparable conditions. However, the consistency of other potentially confounding factors (body weights, food or water intake) was not reported. The study did not report taking measures to minimize the exposure to other plasticizers. Food, tap water, and bedding were not tested for contaminates, and the materials used to dispense water to the animals were not specified. The type of cage animals were housed in was not reported. It is unclear whether the presence of other endocrine disruptor contaminates would impact on the study results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in the results. Quantitative outcomes were reported for each group for most outcomes (colon weight not reported). The number of animals used for determining cytokine levels was 4-6/group; study authors did not provide an explanation. This is not expected to significantly impact results.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.
Health Outcome(s) and Reported Health Effect(s):	Immune/Hematological-Cytokine levels in colon via array (40 different cytokines) and ELISA (IL-1RA and CXCL12). Immunohistochemistry for goblet cells (MUC2). RNA levels of colon mRNA levels of immune and immune-related factors including: mucins (Muc1, 2, 3a, and 4), toll-like receptors (Trl4and 5), and specialized epithelial cells (ChgA [mature enteroendocrine cells], Lgr5 [stem cells], Lyz1 [Paneth cell marker], Cd24a [epithelial cell marker for cells located at bottom of intestinal crypts], and Vil1 [epithelium border]).
Duration and Exposure Route:	Oral-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11151638

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma-Aldrich) 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 at the time of purchase. There is no indication that the test substance was verified by the performing laboratory. Details on the preparation are provided in the cited reference (Chiu et al. 2020). No information was provided on the method used for mixing or how far in advance solutions were made. Storage or stability of the test solutions were not provided. Animals were dosed by gently pipetting chemical into the mouth (volume not reported). Dosing occurred at the same time each day. Doses were reported in mg/kg-day. It was not specified whether the doses were adjusted daily based on body weight, however, the exposure period was only 2 weeks. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The estrous cycle was monitored, and animals were sacrificed when they were in diestrus. This is appropriate since the authors were looking at estradiol levels; however, it also meant animals were not all exposed the same number of days. Exposure durations varied from 10-14 days- the exact duration within and across groups was not reported).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species and sample size are appropriate to evaluate outcomes of interest. A wide range of doses were studied (0.02 – 200 mg/kg/day). Doses were chosen to both mimic environmentally relevant exposure in humans (0.02 and 0.2 mg/kg/day) and to understand toxicological dose responses (2, 20, and 200 mg/kg/day). 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	Data were fully reported as means +/- SEM for most endpoints. Immunohistochemistry results were reported as both images and quantified values. Data on cytokine array was not fully reported. Statistical analysis was reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.
Health Outcome(s) and Reported Health Effect(s):	Gastrointestinal-Distal colon weight, immunohistochemistry for proliferation (Ki67).
Duration and Exposure Route:	Oral-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11151638

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information were reported. The test material was identified as di-isononyl phthalate (DINP) (CAS no. 2855-3-12-0) and the source was reported. Other reported information included details on the test model (species, strain, source, and age); animal husbandry (food and water availability, temperature, humidity, light cycle); exposure details, experimental design, number of animals per group, endpoint evaluation methods, and results for the endpoint of interest. Missing information included the test chemical purity, number of animals/ per cage, and initial body weights.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors did not report how 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 evaluated were not subjective and used standard kits (e.g., ELISA, immunohistochemistry).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. No positive control was included nor required for the study. Based on the data provided, study groups were maintained, exposed, and evaluated under comparable conditions. However, the consistency of other potentially confounding factors (body weights, food or water intake) was not reported. The study did not report taking measures to minimize the exposure to other plasticizers. Food, tap water, and bedding were not tested for contaminates, and the materials used to dispense water to the animals were not specified. The type of cage animals were housed in was not reported. It is unclear whether the presence of other endocrine disruptor contaminates would impact on the study results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in the results. Quantitative outcomes were reported for each group for most outcomes (colon weight not reported). The number of animals used for determining cytokine levels was 4-6/group; study authors did not provide an explanation. This is not expected to significantly impact results.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Chiu, K., Bashir, S. T., Chiu, J., Nowak, R. A., Flaws, J. A. (2021). The Impact of Di-Isononyl Phthalate Exposure on Specialized Epithelial Cells in the Colon. Toxicological Sciences 184(1):142-153.
Health Outcome(s) and Reported Health Effect(s):	Gastrointestinal-Distal colon weight, immunohistochemistry for proliferation (Ki67).
Duration and Exposure Route:	Oral-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11151638

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma-Aldrich) 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 at the time of purchase. There is no indication that the test substance was verified by the performing laboratory. Details on the preparation are provided in the cited reference (Chiu et al. 2020). No information was provided on the method used for mixing or how far in advance solutions were made. Storage or stability of the test solutions were not provided. Animals were dosed by gently pipetting chemical into the mouth (volume not reported). Dosing occurred at the same time each day. Doses were reported in mg/kg-day. It was not specified whether the doses were adjusted daily based on body weight, however, the exposure period was only 2 weeks. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The estrous cycle was monitored, and animals were sacrificed when they were in diestrus. This is appropriate since the authors were looking at estradiol levels; however, it also meant animals were not all exposed the same number of days. Exposure durations varied from 10-14 days- the exact duration within and across groups was not reported).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species and sample size are appropriate to evaluate outcomes of interest. A wide range of doses were studied (0.02 – 200 mg/kg/day). Doses were chosen to both mimic environmentally relevant exposure in humans (0.02 and 0.2 mg/kg/day) and to understand toxicological dose responses (2, 20, and 200 mg/kg/day). 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	Colon weights were not reported. Data were reported as means +/- SEM for proliferation. Statistical analysis was reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Chiu, K., Bashir, S. T., Nowak, R. A., Mei, W., Flaws, J. A. (2020). Subacute exposure to di-isononyl phthalate alters the morphology, endocrine function, and immune system in the colon of adult female mice. Scientific Reports 10(1):18788-18788.
Health Outcome(s) and Reported Health Effect(s):	Gastrointestinal-Gross measurements of the colon , Histological analysis , Colon Hormone level , Gene expression
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978425

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The chemical name (Di-isononyl phthalate, DINP), CASRN# (28553-12-0), the exposure concentration of five groups (0.02, 0.2, 2, 20, and 200mg/kg) and corn oil control, the duration of exposure (10-14 days; additional 4 days until diestrus), and the route of exposure (oral) were provided. The test animal species (mice), strain (CD-1), sex (female), animal supplier (Charles River, Wilmington, MA). Animal age at the time of exposure was specified (2 month old). Information on animal husbandry; temperature (21±C°), humidity (50±20%), and 12 hours light/dark cycle were reported. The number of animals per cage (3 per cage and were allowed for 7 days of acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported. Sample size (36 total, 6/group) was provided, the purity was not reported, and body weights at initiation of dosing were not reported, which has no effect on outcomes.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	This study is considered low for metric 2.1. Animals were selected randomly; no other method of randomization were provided.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Colon histology was randomized and graded without knowledge of treatment group.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The use of corn oil vehicle control was reported. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Qualitative and quantitative results were reported for all outcomes described in the methods (hormonal level, gene expression, and histopathological examination). The study reported the number of the animals (6/group).
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Chiu, K., Bashir, S. T., Nowak, R. A., Mei, W., Flaws, J. A. (2020). Subacute exposure to di-isononyl phthalate alters the morphology, endocrine function, and immune system in the colon of adult female mice. Scientific Reports 10(1):18788-18788.
Health Outcome(s) and Reported Health Effect(s):	Gastrointestinal-Gross measurements of the colon , Histological analysis , Colon Hormone level , Gene expression
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978425

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP), CASRN#, source. The authors indicated the 200mg/kg DINP stock solution was made by dilution in corn oil with (density 0.972 g/mL at 25 °C). The other doses were created from the stocking solution by serial dilutions (control group exposed to corn oil). No purity was reported, nor independent analytical verification of the test article purity performed.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study intended to measure the oral impact of DINP on GIT toxicity, the route of exposure was appropriate for the study type and outcomes (e.g., gene expression, histopathology). The exposure frequency was not clearly presented, however, study duration of exposure was reported and may not have impact on the outcome.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal selected, species, strain sex, life-stage (mice- CD-1, 2-month-old female) was relevant to evaluation of the outcomes. Sample size (n=6/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology addressed the proposed outcomes (e.g., histology finding evaluated).
	Metric 9:	Results presentation	High	Quantitative and qualitative data was presented in this study as means ± standard error of the means (SEM). The authors concluded that acute exposure to DINP increases colonic damage, decreases hormonal level, alter cytokine levels, and dysregulates expression of cell cycle regulated genes downregulates expression of tight junction, in adult female CD-1mice.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Clewell, R. A., Sochaski, M., Edwards, K., Creasy, D. M., Willson, G., Andersen, M. E. (2013). Disposition of diisononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats. Reproductive Toxicology 35(1):56–69.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weight-Hepatic/Liver-Maternal liver weight-Reproductive/Developmental-Fetal weight, testis testosterone level, AGD, and histology on testis
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 12-19)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325350

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. The test substance was identified as diisonoyl phthalate (DiNP), CAS# 68515-48-0. The source and purity (90%) were reported. Test animal species, strain, sex, and source were reported. Age of animals and initial body weight 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	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 for non-subjective endpoints (body weight, organ weight, AGD, testosterone levels). Histological slides were evaluated using a semi-blinded method of evaluation. Initial review was done with knowledge of positive and negative control groups, followed by a blinded evaluation (without knowledge of treatment group).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Husbandry conditions were reported and similar between groups. A negative control group was 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 substantially 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 results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	The study does not report any deaths and there is no indication that any animals were removed from analysis.

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Study Citation:	Clewell, R. A., Sochaski, M., Edwards, K., Creasy, D. M., Willson, G., Andersen, M. E. (2013). Disposition of diiosononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats. Reproductive Toxicology 35(1):56–69.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weight-Hepatic/Liver-Maternal liver weight-Reproductive/Developmental-Fetal weight, testis testosterone level, AGD, and histology on testis
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 12-19)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325350

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	Test substance was reported to be 90% pure. Stock solutions were prepared and concentrations verified by gas chromatography-mass spectrometry. Stock solutions were stable for 2 weeks (dosing solutions were used within 2 weeks of preparation). Maternal body weights were measured daily prior to dosing and entered into Provantis for calculation of administered dose volume. Gavage volume was appropriate (1 ml/kg).
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency and duration of exposure were appropriate for study's aim.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Endpoints were sensitive to outcomes of interest. The number of exposure groups and doses were adequate to identify a full range of responses.
	Metric 9:	Results presentation	High	Data were fully reported with mean and standard error. Statistical analysis was appropriate. The litter was used as the statistical unit.

Additional Comments: None

Overall Quality Determination High

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) and Reported Health Effect(s):	Mortality-Mortality
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Reporting 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 exposure, 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, ventilation, 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 and 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 / 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				

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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) and Reported Health Effect(s):	Mortality-Mortality
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl 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 Methods Sensitivity				
	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 information 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 Measures and Results 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 Quality Determination 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 Issues 72(21-22):1446-1454.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Reporting 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 exposure, 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, ventilation, 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 and 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 / 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				
	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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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) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	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 information 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 Measures and Results 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 Quality Determination 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 Issues 72(21-22):1446-1454.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Reporting 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 exposure, 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, ventilation, 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 and 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 / 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				

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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) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl 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 Methods Sensitivity				
	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 information 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 Measures and Results Display				
	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, although there is not enough information to determine if they were evaluated consistently. The outcome methodology only partially addressed the outcome of interests as histopathology and functionality were not evaluated.
	Metric 9:	Results presentation	Medium	Data were presented quantitatively along with the appropriate statistical analysis. Urinalysis data was not reported.

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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) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	697382

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination **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 Issues 72(21-22):1446-1454.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testis and epididymis weights, sperm count and motility
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Reporting 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 exposure, 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, ventilation, 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 and 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 / 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				

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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) and Reported Health Effect(s):	Reproductive/Developmental-Testis and epididymis weights, sperm count and motility
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-4-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl 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 Methods Sensitivity				
	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 information 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 Measures and Results 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	High	Data were presented quantitatively along with the appropriate statistical analysis.

Additional Comments: None

Overall Quality Determination — Medium

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) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	673292

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 ≥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 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	Husbandry conditions were reported and similar between groups. Negative and positive 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 substantially 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 results.
Domain 4: Selective Reporting and Attrition				
	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 Methods Sensitivity				

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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) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	673292

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	Purity was reported to be ≥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.
	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 Measures and Results 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

Overall Quality Determination Medium

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) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	673292

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 ≥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 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 / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Husbandry conditions were reported and similar between groups. Negative and positive 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 substantially 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 results.
Domain 4: Selective Reporting and Attrition				
	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 Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	Purity was reported to be ≥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., 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) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-10-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	673292

Domain	Metric		Rating	Comments
	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 Measures and Results 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 Quality Determination Medium

Study Citation:	Liang, F., Yan, B. (2020). Oxidative damage in the liver and kidney induced by dermal exposure to diisononyl phthalate in Balb/c mice. Toxicology and Industrial Health 36(1):30-40.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-body weight-Hepatic/Liver-organ weight, organ coefficient, ROS, MDA, GSH, DPC coefficient and histology-Renal/Kidney-organ weight, organ coefficient, ROS, MDA, GSH, DPC coefficient and histology-Skin/Connective Tissue-histology
Duration and Exposure Route:	Dermal-Duration: Short-term (>1-30 days)-7-24-28-day(s)
Species:	Mouse-Balb/c - [mouse]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978423

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The chemical name (Di-isononyl phthalate, DINP), CASRN# was not reported, t), the purity (99.6%), the exposure concentration of five groups (0.02, 0.2, 2, 20, and 200mg/kg) and saline control, the duration of exposure (continuous exposure up to 28 days), and the route of exposure (dermal) were provided. The test animal species (mice), strain (SPF), sex (male), animal supplier (Wuhan, Hubei province, China), body weights at initiation of dosing were reported (22-25g). Animal age at the time of exposure was specified (5 weeks old). Information on animal husbandry; temperature (20-25C°), humidity (50-70 humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (all animal housed under SPF condition during the 5 days of acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (42, 7/group) was provided
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered low for metric 2.1. Animals were selected randomly; no other method of randomization were provided.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were reported; histology was examined by two experienced pathologists in a blinded manner.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	The use of negative control was reported. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Qualitative and quantitative results were reported for all outcomes described in the methods (Body weight, organ weight, organ coefficient and histopathological examination) The study reported the number of the animals (6/group for body weight and organ coefficients) to specific animal dose groups). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Liang, F., Yan, B. (2020). Oxidative damage in the liver and kidney induced by dermal exposure to diisononyl phthalate in Balb/c mice. Toxicology and Industrial Health 36(1):30-40.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-body weight-Hepatic/Liver-organ weight, organ coefficient, ROS, MDA, GSH, DPC coefficient and histology-Renal/Kidney-organ weight, organ coefficient, ROS, MDA, GSH, DPC coefficient and histology-Skin/Connective Tissue-histology
Duration and Exposure Route:	Dermal-Duration: Short-term (>1-30 days)-7-24-28-day(s)
Species:	Mouse-Balb/c - [mouse]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978423

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP), source and 99.6% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the DINP solutions were prepared using normal saline; 20 mL of the solution was applied evenly to the back of each mouse (the control group exposed to equal volume of saline. Test animals were divided into 5 groups at 5 dose levels and untreated control. As indicated by the authors, seven mice were used in each group to minimize the number of experimental animal while ensuring the statistical validity of the results: no significant impact on the outcome of the study
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study intended to measure the dermal effect of DINP on liver and kidney toxicity, the route of exposure was appropriate for the study type and outcomes (e.g., body weight, histopathology) and the exposure frequency and duration was adequately stated.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The test animal selected, species, strain sex, life-stage (mice, 5 weeks old male) was relevant to evaluation of the outcomes. Sample size (n=7/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology addressed the proposed outcomes (e.g., organ weight and histology finding evaluated). Minor limitations were identified in the sampling of the outcomes (e.g., 6 animal/group for analysis rather than the 7 previously stated).
	Metric 9:	Results presentation	Medium	The study considered med for each outcome. Quantitative data (means and SD) are provided. Incidence data from histological examinations are reported

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-1-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784618

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified as di(2-ethylhexyl)phthalate. No CASRN was provided. 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. Husbandry conditions (temperature, humidity, and light cycle) were not reported. Animals were housed 3/cage. Feed and water were available ad libitum. Dose levels (ppm), duration, 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 concentrations were reported; however, actual doses were not. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and 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 / 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 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				

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Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-1-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784618

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 Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	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.
	Metric 7:	Exposure timing, frequency, and duration	High	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 Measures and Results 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 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 ± 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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Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-1-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784618

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

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) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight-Renal/Kidney-organ weight, renal biomarkers for oxidative stress (ROS, MDA, GSH), inflammatory cytokines (TNF-a and IL-6)
Duration and Exposure Route:	Oral-Gavage-Duration: Subchronic (>30-90 days)-7-5-week(s)
Species:	Mouse-ICR - [mouse]-Male
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	7978408

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 and 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: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A vehicle control groups was included. No effect of test substance palatability in dietary 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 Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative or qualitative results were reported for all prespecified outcomes, no animal attrition identified.
Domain 5: Exposure Methods Sensitivity				
	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.

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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) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight-Renal/Kidney-organ weight, renal biomarkers for oxidative stress (ROS, MDA, GSH), inflammatory cytokines (TNF-a and IL-6)
Duration and Exposure Route:	Oral-Gavage-Duration: Subchronic (>30-90 days)-7-5-week(s)
Species:	Mouse-ICR - [mouse]-Male
Chemical:	Diisononyl 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 Measures and Results 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			

Overall Quality Determination Medium

Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain, food consumption-Mortality-Death, survival-Immune/Hematological-Hematological parameters (erythrocytes, hemoglobin, total and differential leukocytes, platelets, hematocrit, erythrocyte morphology), gross pathology and histopathology: blood smear, bone marrow smear, lymph nodes, spleen-Renal/Kidney-Urinalysis (pH, ketones, protein, bilirubin, occult blood, urobilinogen, glucose, urobilinogen, gross appearance, specific gravity), organ weights: kidney, gross pathology and histopathology: kidneys, urinary bladder, uterus (corpus and cervix uteri)-Cardiovascular-Organ weights: heart, gross pathology and histopathology: aorta, heart-Gastrointestinal-gross pathology and histopathology: esophagus, intestine, cecum, colon, duodenum, ileum, rectum, salivary gland, seminal vesicles, stomach, tongue-Lung/Respiratory-gross pathology and histopathology: larynx, lungs, trachea-Reproductive/Developmental-organ weights: ovaries, testes, epididymides, mammary gland, ovaries, prostate, testes, epididymides-Neurological/Behavioral-Organ weights: brain, gross pathology and histopathology: brain, spinal cord (cervical, lumbar)-Musculoskeletal-organ weights: biceps femoris, gross pathology and histopathology: bone with marrow, skeletal muscle-Skin/Connective Tissue-gross pathology and histopathology: skin-Other (please specify below) (Endocrine)-Organ weights: adrenals, pituitary, thyroid/parathyroids, gross pathology and histopathology: adrenals, pancreas, pituitary, thymus, thyroid/parathyroids-Other (please specify below) (Clinical Signs)-Clinical Signs-Cancer/Carcinogenesis-Neoplastic lesions
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	Test substance identity is determined via CASRN (a proprietary chemical name Santicizer 900 is reported rather than the actual chemical name), and test substance source and purity are reported. Test animal species, strain, sex, source, starting age and starting body weights were all reported. Animal housing conditions that were reported included number of animals per cage, food and water availability, and light/dark cycle. Exposure methods and outcome assessment methods were reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to groups using a computer program that ensured that body weights were distributed equally between groups.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No methods to reduce observational bias were described, but endpoints of interest were objective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Due to the dietary and ad libitum nature of the exposure, food palatability is a possible concern, but no significant differences in food consumption were reported, so any impact on palatability is not likely to have a major influence on the results. Other factors relevant for determining confounding (such as body weights and body weight gain) are reported. An appropriate negative control is reported, and there are no concerns with control response.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain, food consumption-Mortality-Death, survival-Immune/Hematological-Hematological parameters (erythrocytes, hemoglobin, total and differential leukocytes, platelets, hematocrit, erythrocyte morphology), gross pathology and histopathology: blood smear, bone marrow smear, lymph nodes, spleen-Renal/Kidney-Urinalysis (pH, ketones, protein, bilirubin, occult blood, urobilinogen, glucose, urobilinogen, gross appearance, specific gravity), organ weights: kidney, gross pathology and histopathology: kidneys, urinary bladder, uterus (corpus and cervix uteri)-Cardiovascular-Organ weights: heart, gross pathology and histopathology: aorta, heart-Gastrointestinal-gross pathology and histopathology: esophagus, intestine, cecum, colon, duodenum, ileum, rectum, salivary gland, seminal vesicles, stomach, tongue-Lung/Respiratory-gross pathology and histopathology: larynx, lungs, trachea-Reproductive/Developmental-organ weights: ovaries, testes, epididymides, mammary gland, ovaries, prostate, testes, epididymides-Neurological/Behavioral-Organ weights: brain, gross pathology and histopathology: brain, spinal cord (cervical, lumbar)-Musculoskeletal-organ weights: biceps femoris, gross pathology and histopathology: bone with marrow, skeletal muscle-Skin/Connective Tissue-gross pathology and histopathology: skin-Other (please specify below) (Endocrine)-Organ weights: adrenals, pituitary, thyroid/parathyroids, gross pathology and histopathology: adrenals, pancreas, pituitary, thymus, thyroid/parathyroids-Other (please specify below) (Clinical Signs)-Clinical Signs-Cancer/Carcinogenesis-Neoplastic lesions
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	There are no concerns regarding attrition, all animals are accounted for in the results and individual animal data are included in the results. All prespecified outcomes are reported in the results and there are no concerns regarding omission bias.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The authors indicate that test substance purity was analytically confirmed and did not contain any contaminants. Regarding test substance preparation, the authors also conducted tests on to confirm the amount and homogeneity of the test substance in feed, and prepared diets weekly to ensure there were no issues with exposure administration from test substance stability. Storage conditions were not described, but the test substance is generally stable at room temperature. There are minor uncertainties with administered dose derivation, with nominal doses used to calculate test substance intake. Authors report actual concentrations were within 15% of nominal.
	Metric 7:	Exposure timing, frequency, and duration	High	The duration and frequency of the exposure was sensitive for outcomes of interest and was consistent between the different groups of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The chosen species and strain and sample size are appropriate for chronic toxicity studies. There are no concerns regarding the timing of endpoint assessment. The outcome methodology was sensitive to detect effects of interest. Dose and concentration spacing is adequate, but an additional lower dose would need to be included in order to derive a study-wide NOAEL. There are also minor concerns that histopathology was only evaluated in control and high dose animals.
	Metric 9:	Results presentation	Medium	Statistical analysis is described, but some relevant information (such as the type of test performed) are not described in adequate detail, but this missing information is unlikely to have a major impact on the interpretation of the results. There is full quantitative presentation of the results and qualitative description of some results in the text. Some outcomes are only described in individual animal data in the appendices.

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Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain, food consumption-Mortality-Death, survival-Immune/Hematological-Hematological parameters (erythrocytes, hemoglobin, total and differential leukocytes, platelets, hematocrit, erythrocyte morphology), gross pathology and histopathology: blood smear, bone marrow smear, lymph nodes, spleen-Renal/Kidney-Urinalysis (pH, ketones, protein, bilirubin, occult blood, urobilinogen, glucose, urobilinogen, gross appearance, specific gravity), organ weights: kidney, gross pathology and histopathology: kidneys, urinary bladder, uterus (corpus and cervix uteri)-Cardiovascular-Organ weights: heart, gross pathology and histopathology: aorta, heart-Gastrointestinal-gross pathology and histopathology: esophagus, intestine, cecum, colon, duodenum, ileum, rectum, salivary gland, seminal vesicles, stomach, tongue-Lung/Respiratory-gross pathology and histopathology: larynx, lungs, trachea-Reproductive/Developmental-organ weights: ovaries, testes, epididymides, mammary gland, ovaries, prostate, testes, epididymides-Neurological/Behavioral-Organ weights: brain, gross pathology and histopathology: brain, spinal cord (cervical, lumbar)-Musculoskeletal-organ weights: biceps femoris, gross pathology and histopathology: bone with marrow, skeletal muscle-Skin/Connective Tissue-gross pathology and histopathology: skin-Other (please specify below) (Endocrine)-Organ weights: adrenals, pituitary, thyroid/parathyroids, gross pathology and histopathology: adrenals, pancreas, pituitary, thymus, thyroid/parathyroids-Other (please specify below) (Clinical Signs)-Clinical Signs-Cancer/Carcinogenesis-Neoplastic lesions
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric	Rating	Comments

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Blood serum chemistry (total protein, triglycerides, blood urea nitrogen, albumin, fasting glucose, globulin, glutamic pyruvic transaminase, A/G Ratio, Glutamic oxaloacetic transaminase, sodium, alkaline phosphatase, potassium, cholesterol, calcium), organ weights: liver, gross pathology and histopathology: liver
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	Test substance identity is determined via CASRN (a proprietary chemical name Santicizer 900 is reported rather than the actual chemical name), and test substance source and purity are reported. Test animal species, strain, sex, source, starting age and starting body weights were all reported. Animal housing conditions that were reported included number of animals per cage, food and water availability, and light/dark cycle. Exposure methods and outcome assessment methods were reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to groups using a computer program that ensured that body weights were distributed equally between groups.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No methods to reduce observational bias were described, but endpoints of interest were objective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Due to the dietary and ad libitum nature of the exposure, food palatability is a possible concern, but no significant differences in food consumption were reported, so any impact on palatability is not likely to have a major influence on the results. Other factors relevant for determining confounding (such as body weights and body weight gain) are reported. An appropriate negative control is reported, and there are no concerns with control response.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	There are no concerns regarding attrition, all animals are accounted for in the results and individual animal data are included in the results. All prespecified outcomes are reported in the results and there are no concerns regarding omission bias.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The authors indicate that test substance purity was analytically confirmed and did not contain any contaminants. Regarding test substance preparation, the authors also conducted tests on to confirm the amount and homogeneity of the test substance in feed, and prepared diets weekly to ensure there were no issues with exposure administration from test substance stability. Storage conditions were not described, but the test substance is generally stable at room temperature. There are minor uncertainties with administered dose derivation, with nominal doses used to calculate test substance intake. Authors report actual concentrations were within 15% of nominal.

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Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Blood serum chemistry (total protein, triglycerides, blood urea nitrogen, albumin, fasting glucose, globulin, glutamic pyruvic transaminase, A/G Ratio, Glutamic oxaloacetic transaminase, sodium, alkaline phosphatase, potassium, cholesterol, calcium), organ weights: liver, gross pathology and histopathology: liver
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The duration and frequency of the exposure was sensitive for outcomes of interest and was consistent between the different groups of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The chosen species and strain and sample size are appropriate for chronic toxicity studies. There are no concerns regarding the timing of endpoint assessment. The outcome methodology was sensitive to detect effects of interest. Dose and concentration spacing is adequate, but an additional lower dose would need to be included in order to derive a study-wide NOAEL. Histopathology was conducted for all dose groups in liver tissue.
	Metric 9:	Results presentation	Medium	Statistical analysis is described, but some relevant information (such as the type of test performed) are not described in adequate detail, but this missing information is unlikely to have a major impact on the interpretation of the results. There is full quantitative presentation of the results and qualitative description of some results in the text. Some outcomes are only described in individual animal data in the appendices.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Ocular)-gross pathology and histopathology: eyes with optic nerve, harderian gland, ocular examination.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	Test substance identity is determined via CASRN (a proprietary chemical name Santicizer 900 is reported rather than the actual chemical name), and test substance source and purity are reported. Test animal species, strain, sex, source, starting age and starting body weights were all reported. Animal housing conditions that were reported included number of animals per cage, food and water availability, and light/dark cycle. Exposure methods and outcome assessment methods were reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to groups using a computer program that ensured that body weights were distributed equally between groups.
	Metric 3:	Observational Bias / Blinding Changes	Low	For ophthalmic observations a separate investigator was used to assess this endpoint, but the study report does not clarify whether this investigator was aware of study group designation during ophthalmic observations.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Due to the dietary and ad libitum nature of the exposure, food palatability is a possible concern, but no significant differences in food consumption were reported, so any impact on palatability is not likely to have a major influence on the results. Other factors relevant for determining confounding (such as body weights and body weight gain) are reported. An appropriate negative control is reported, and there are no concerns with control response.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	There are no concerns regarding attrition, all animals are accounted for in the results and individual animal data are included in the results. All prespecified outcomes are reported in the results and there are no concerns regarding omission bias.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The authors indicate that test substance purity was analytically confirmed and did not contain any contaminants. Regarding test substance preparation, the authors also conducted tests on to confirm the amount and homogeneity of the test substance in feed, and prepared diets weekly to ensure there were no issues with exposure administration from test substance stability. Storage conditions were not described, but the test substance is generally stable at room temperature. There are minor uncertainties with administered dose derivation, with nominal doses used to calculate test substance intake. Authors report actual concentrations were within 15% of nominal.

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Study Citation:	Bio/dynamics, (1987). A chronic toxicity carcinogenicity feeding study in rats with Santicizer 900 with cover letter dated 06/05/87.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Ocular)-gross pathology and histopathology: eyes with optic nerve, harderian gland, ocular examination.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-2-year(s)
Species:	Rat-Other (Sprague-Dawley CD)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	679889

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The duration and frequency of the exposure was sensitive for outcomes of interest and was consistent between the different groups of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The chosen species and strain and sample size are appropriate for chronic toxicity studies. There are no concerns regarding the timing of endpoint assessment. The outcome methodology was sensitive to detect effects of interest. Dose and concentration spacing is adequate, but an additional lower dose would need to be included in order to derive a study-wide NOAEL. There are also minor concerns that histopathology was only evaluated in control and high dose animals.
	Metric 9:	Results presentation	Medium	Statistical analysis is described, but some relevant information (such as the type of test performed) are not described in adequate detail, but this missing information is unlikely to have a major impact on the interpretation of the results. There is full quantitative presentation of the results and qualitative description of some results in the text. Some outcomes are only described in individual animal data in the appendices.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Neurological/Behavioral-organ weights: brain with stem weight; gross examination of brain and spinal cord ; behavioral-related clinical signs; histopathology: brain with stem, peripheral nerve (sciatic), spinal cord.-Renal/Kidney-Organ weight: kidney (paired wt); serum chemistry: calcium, chloride, inorganic phosphorus, potassium, urea nitrogen, creatinine; urinalysis parameters: volume, osmolality, electrolytes, creatinine, creatinine clearance, appearance, bilirubin, glucose, ketones, occult blood, pH, specific gravity, urobilinogen, microscopic examination of sediment; gross necropsy; histopathology: kidney, urinary bladder-Reproductive/Developmental-Organ weights: testis (with epididymides) and uterus; gross necropsy; histopathology: seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries, prostate-Lung/Respiratory-Lung weight; gross examination of nasal cavity and paranasal sinuses; gross necropsy (nasal cavity and paranasal sinuses); histopathology: lung, trachea; respiratory-related clinical signs-Cancer/Carcinogenesis-Microscopic examination for tumors-Cardiovascular-gross necropsy; histopathology: heart, aorta (thoracic)-Gastrointestinal-Gross necropsy; histopathology: large intestine (colon, cecum, rectum), small intestine (duodenum, jejunum, ileum), stomach (cardia, fundus, pylorus), esophagus, salivary glands (mandibular).-Skin/Connective Tissue-Clinical observations: skin- and tissue-related; gross examination (external surface of body).-Other (please specify below) (General gross necropsy)-gross necropsy: all orifices, carcass, cranial cavity, thoracic, abdominal, and pelvic cavities/viscera-Other (please specify below) (Non-specific targeted clinical signs)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)-Musculoskeletal-gross necropsy; histopathology: femur with marrow and joint and skeletal muscle (thigh); Clinical signs (malocclusion)-Ocular/Sensory-gross necropsy; histopathology: eyes with optic nerves, exorbital lacrimal glands; clinical observations.-Other (please specify below) (Endocrine)-Gross necropsy; histopathology: adrenal gland, pancreas, pituitary.-Thyroid-Histopathology: thyroid with parathyroid.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported, including details of the test substance (DINP, source, purity), animal model (species, strain, sex, age, source, starting body weights), animal husbandry conditions (housing/cage details, animals/cage, food and water availability, room temperature, humidity, light cycle, air changes), the method of administration, and experimental design details. Quantitative results were reported for all of the endpoints described.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated into study groups using a computerized-weight-randomization program. Animals selected for interim sacrifices were selected from animals with the highest animal identification numbers within each group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified; however, the endpoints were either simple or not subjective (e.g., mortality, organ weight measurements), or blinding is not recommended (initial histopathology), or required in current guidelines for the endpoints (clinical observations and gross necropsy).
Domain 3: Confounding / Variable Control				

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Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Neurological/Behavioral-organ weights: brain with stem weight; gross examination of brain and spinal cord ; behavioral-related clinical signs; histopathology: brain with stem, peripheral nerve (sciatic), spinal cord.-Renal/Kidney-Organ weight: kidney (paired wt); serum chemistry: calcium, chloride, inorganic phosphorus, potassium, urea nitrogen, creatinine; urinalysis parameters: volume, osmolality, electrolytes, creatinine, creatinine clearance, appearance, bilirubin, glucose, ketones, occult blood, pH, specific gravity, urobilinogen, microscopic examination of sediment; gross necropsy; histopathology: kidney, urinary bladder-Reproductive/Developmental-Organ weights: testis (with epididymides) and uterus; gross necropsy; histopathology: seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries, prostate-Lung/Respiratory-Lung weight; gross examination of nasal cavity and paranasal sinuses; gross necropsy (nasal cavity and paranasal sinuses); histopathology: lung, trachea; respiratory-related clinical signs-Cancer/Carcinogenesis-Microscopic examination for tumors-Cardiovascular-gross necropsy; histopathology: heart, aorta (thoracic)-Gastrointestinal-Gross necropsy; histopathology: large intestine (colon, cecum, rectum), small intestine (duodenum, jejunum, ileum), stomach (cardia, fundus, pylorus), esophagus, salivary glands (mandibular).-Skin/Connective Tissue-Clinical observations: skin- and tissue-related; gross examination (external surface of body).-Other (please specify below) (General gross necropsy)-gross necropsy: all orifices, carcass, cranial cavity, thoracic, abdominal, and pelvic cavities/viscera-Other (please specify below) (Non-specific targeted clinical signs)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)-Musculoskeletal-gross necropsy; histopathology: femur with marrow and joint and skeletal muscle (thigh); Clinical signs (malocclusion)-Ocular/Sensory-gross necropsy; histopathology: eyes with optic nerves, exorbital lacrimal glands; clinical observations.-Other (please specify below) (Endocrine)-Gross necropsy; histopathology: adrenal gland, pancreas, pituitary.-Thyroid-Histopathology: thyroid with parathyroid.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	High	Negative controls were fed basal diets. The negative control responses were appropriate for all of the outcomes specified. Food was analyzed for contamination from other organophosphates, and water was analyzed for contaminates. Animals were housed in stainless-steel, wire-mesh cages. There were no significant differences in food consumption observed between the DINP treated mice and the control and no mention of palatability issues. No confounding variables that could impact the results of the study were identified.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative results for all endpoints were reported. No animal attrition unrelated to treatment was described. All of the animals were accounted for and sample sizes were clearly reported. There was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Neurological/Behavioral-organ weights: brain with stem weight; gross examination of brain and spinal cord ; behavioral-related clinical signs; histopathology: brain with stem, peripheral nerve (sciatic), spinal cord.-Renal/Kidney-Organ weight: kidney (paired wt); serum chemistry: calcium, chloride, inorganic phosphorus, potassium, urea nitrogen, creatinine; urinalysis parameters: volume, osmolality, electrolytes, creatinine, creatinine clearance, appearance, bilirubin, glucose, ketones, occult blood, pH, specific gravity, urobilinogen, microscopic examination of sediment; gross necropsy; histopathology: kidney, urinary bladder-Reproductive/Developmental-Organ weights: testis (with epididymides) and uterus; gross necropsy; histopathology: seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries, prostate-Lung/Respiratory-Lung weight; gross examination of nasal cavity and paranasal sinuses; gross necropsy (nasal cavity and paranasal sinuses); histopathology: lung, trachea; respiratory-related clinical signs-Cancer/Carcinogenesis-Microscopic examination for tumors-Cardiovascular-gross necropsy; histopathology: heart, aorta (thoracic)-Gastrointestinal-Gross necropsy; histopathology: large intestine (colon, cecum, rectum), small intestine (duodenum, jejunum, ileum), stomach (cardia, fundus, pylorus), esophagus, salivary glands (mandibular).-Skin/Connective Tissue-Clinical observations: skin- and tissue-related; gross examination (external surface of body).-Other (please specify below) (General gross necropsy)-gross necropsy: all orifices, carcass, cranial cavity, thoracic, abdominal, and pelvic cavities/viscera-Other (please specify below) (Non-specific targeted clinical signs)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)-Musculoskeletal-gross necropsy; histopathology: femur with marrow and joint and skeletal muscle (thigh); Clinical signs (malocclusion)-Ocular/Sensory-gross necropsy; histopathology: eyes with optic nerves, exorbital lacrimal glands; clinical observations.-Other (please specify below) (Endocrine)-Gross necropsy; histopathology: adrenal gland, pancreas, pituitary.-Thyroid-Histopathology: thyroid with parathyroid.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test material, source, lot number, purity, and storage details were reported. Other details (methods of synthesis, stability, composition etc.) were reported to be on file with the sponsor, but not provided in the study report. The test material was not independently analyzed by the performing laboratory. Comprehensive details of the test diet preparations were provided. Diets were prepared fresh weekly and stored refrigerated in non-plastic containers. They were mixed to homogeneity and stability was tested. Concentrations of the test material in the diets were measured using reverse-phase, high performance liquid chromatography during weeks 1, 13, 26, 52, 78, and 104. Target concentrations in food and calculated average daily doses were provided. Concentration analysis indicated concentrations remained within 92 – 112% of the targets, which was considered by the authors to be acceptable. Individual food intake and body weight data were provided in a dietary study and average daily doses were calculated. The route of exposure was justified by the authors.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for an oncogenicity study and were consistent across groups. Mice were exposed daily, via the diet, for at least 104 weeks.

Domain 6: Outcome Measures and Results Display

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Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Neurological/Behavioral-organ weights: brain with stem weight; gross examination of brain and spinal cord ; behavioral-related clinical signs; histopathology: brain with stem, peripheral nerve (sciatic), spinal cord.-Renal/Kidney-Organ weight: kidney (paired wt); serum chemistry: calcium, chloride, inorganic phosphorus, potassium, urea nitrogen, creatinine; urinalysis parameters: volume, osmolality, electrolytes, creatinine, creatinine clearance, appearance, bilirubin, glucose, ketones, occult blood, pH, specific gravity, urobilinogen, microscopic examination of sediment; gross necropsy; histopathology: kidney, urinary bladder-Reproductive/Developmental-Organ weights: testis (with epididymides) and uterus; gross necropsy; histopathology: seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries, prostate-Lung/Respiratory-Lung weight; gross examination of nasal cavity and paranasal sinuses; gross necropsy (nasal cavity and paranasal sinuses); histopathology: lung, trachea; respiratory-related clinical signs-Cancer/Carcinogenesis-Microscopic examination for tumors-Cardiovascular-gross necropsy; histopathology: heart, aorta (thoracic)-Gastrointestinal-Gross necropsy; histopathology: large intestine (colon, cecum, rectum), small intestine (duodenum, jejunum, ileum), stomach (cardia, fundus, pylorus), esophagus, salivary glands (mandibular).-Skin/Connective Tissue-Clinical observations: skin- and tissue-related; gross examination (external surface of body).-Other (please specify below) (General gross necropsy)-gross necropsy: all orifices, carcass, cranial cavity, thoracic, abdominal, and pelvic cavities/viscera-Other (please specify below) (Non-specific targeted clinical signs)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)-Musculoskeletal-gross necropsy; histopathology: femur with marrow and joint and skeletal muscle (thigh); Clinical signs (malocclusion)-Ocular/Sensory-gross necropsy; histopathology: eyes with optic nerves, exorbital lacrimal glands; clinical observations.-Other (please specify below) (Endocrine)-Gross necropsy; histopathology: adrenal gland, pancreas, pituitary.-Thyroid-Histopathology: thyroid with parathyroid.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	High	The animal species was justified by the study authors, but no justification for the strain selected was provided. The number of animals per group was appropriate. Detailed methods were described for all of the endpoints evaluated including the timing of outcome assessment and sample sizes. The endpoints were sensitive for the outcomes of interest or are those generally included in oncogenicity studies (e.g, clinical observations, gross necropsy). Histopathology was conducted in the control and high dose group only, except for select tissues (epididymides, uterus, spleen, and kidney), which were examined for all treatment groups. In tissues evaluated only in the control and high dose groups, no significant treatment-related effects were observed in treated animals.
	Metric 9:	Results presentation	High	Individual animal data were provided for all of the endpoints specified. Statistical methods were clearly described and were appropriate for the datasets.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Organ weight: Liver/gallbladder (drained); gross necropsy; histopathology: liver, gallbladder; serum chemistry (albumin, albumin/globulin ratio, AST, ALT, gammy glutamyl transpeptidase, globulin, glucose, total protein, total bilirubin); hepatocellular proliferation rates and biochemical analysis (protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, DNA concentration).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported, including details of the test substance (DINP, source, purity), animal model (species, strain, sex, age, source, starting body weights), animal husbandry conditions (housing/cage details, animals/cage, food and water availability, room temperature, humidity, light cycle, air changes), the method of administration, and experimental design details. Quantitative results were reported for all of the endpoints described.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated into study groups using a computerized-weight-randomization program. Animals selected for interim sacrifices were selected from animals with the highest animal identification numbers within each group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified; however, the endpoints were either simple or not subjective (e.g., mortality, organ weight measurements), or blinding is not recommended (initial histopathology), or required in current guidelines for the endpoints (clinical observations and gross necropsy).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	Negative controls were fed basal diets. The negative control responses were appropriate for all of the outcomes specified. Food was analyzed for contamination from other organophosphates, and water was analyzed for contaminates. Animals were housed in stainless-steel, wire-mesh cages. There were no significant differences in food consumption observed between the DINP treated mice and the control and no mention of palatability issues. No confounding variables that could impact the results of the study were identified.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative results for all endpoints were reported. No animal attrition unrelated to treatment was described. All of the animals were accounted for and sample sizes were clearly reported. There was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Organ weight: Liver/gallbladder (drained); gross necropsy; histopathology: liver, gallbladder; serum chemistry (albumin, albumin/globulin ratio, AST, ALT, gammy glutamyl transpeptidase, globulin, glucose, total protein, total bilirubin); hepatocellular proliferation rates and biochemical analysis (protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, DNA concentration).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test material, source, lot number, purity, and storage details were reported. Other details (methods of synthesis, stability, composition etc.) were reported to be on file with the sponsor, but not provided in the study report. The test material was not independently analyzed by the performing laboratory. Comprehensive details of the test diet preparations were provided. Diets were prepared fresh weekly and stored refrigerated in non-plastic containers. They were mixed to homogeneity and stability was tested. Concentrations of the test material in the diets were measured using reverse-phase, high performance liquid chromatography during weeks 1, 13, 26, 52, 78, and 104. Target concentrations in food and calculated average daily doses were provided. Concentration analysis indicated concentrations remained within 92 – 112% of the targets, which was considered by the authors to be acceptable. Individual food intake and body weight data were provided in a dietary study and average daily doses were calculated. The route of exposure was justified by the authors.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for an oncogenicity study and were consistent across groups. Mice were exposed daily, via the diet, for at least 104 weeks.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species was justified by the study authors, but no justification for the strain selected was provided. Detailed methods were described for all of the endpoints evaluated including the timing of outcome assessment and sample sizes. The sample sizes for the liver cell proliferation and biochemical analyses were small (n = 5); in week 105 sacrifice evaluations, the number of males was further reduced to 3 in the control group and 4 in the high dose group; the number of females was 4 in the high dose group with losses being attributed to due to accidental death or interfering carcinomas. The number of animals per group was appropriate for all other liver endpoints. Hepatocellular proliferation and biochemical analysis were only evaluated in the control and high dose group. Significant effects were observed in the biochemical analysis in the high dose group; since lower dose groups were not included, this precluded the ability to identify NOEL/LOEL values for these endpoints.
	Metric 9:	Results presentation	High	Individual animal data were provided for all of the endpoints specified. Statistical methods were clearly described and were appropriate for the datasets.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Immune/Hematological-Counts of reticulocytes, erythrocytes, leukocytes [corrected and non-corrected], and platelets; hematocrit, hemoglobin, MCH, MCHC, MCV, leukocyte differential and cellular morphology (control and high dose group only), myeloid/erythroid ratio (at necropsy); organ weights (spleen); Gross necropsy; histopathology (spleen, thymus, lymph nodes)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported, including details of the test substance (DINP, source, purity), animal model (species, strain, sex, age, source, starting body weights), animal husbandry conditions (housing/cage details, animals/cage, food and water availability, room temperature, humidity, light cycle, air changes), the method of administration, and experimental design details. Quantitative results were reported for all of the endpoints described.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated into study groups using a computerized-weight-randomization program. Animals selected for interim sacrifices were selected from animals with the highest animal identification numbers within each group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified; however, the endpoints were either simple or not subjective (e.g., mortality, organ weight measurements), or blinding is not recommended (initial histopathology), or required in current guidelines for the endpoints (clinical observations and gross necropsy).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	Negative controls were fed basal diets. The negative control responses were appropriate for all of the outcomes specified. Food was analyzed for contamination from other organophosphates, and water was analyzed for contaminates. Animals were housed in stainless-steel, wire-mesh cages. There were no significant differences in food consumption observed between the DINP treated mice and the control and no mention of palatability issues. No confounding variables that could impact the results of the study were identified.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative results for all endpoints were reported. No animal attrition unrelated to treatment was described. All of the animals were accounted for and sample sizes were clearly reported. There was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Covance Labs, (1998). Support: oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses with cover letter dated 11/18/1998 [2598-105].
Health Outcome(s) and Reported Health Effect(s):	Immune/Hematological-Counts of reticulocytes, erythrocytes, leukocytes [corrected and non-corrected], and platelets; hematocrit, hemoglobin, MCH, MCHC, MCV, leukocyte differential and cellular morphology (control and high dose group only), myeloid/erythroid ratio (at necropsy); organ weights (spleen); Gross necropsy; histopathology (spleen, thymus, lymph nodes)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Mouse-B6C3F1 - [mouse]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325481

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test material, source, lot number, purity, and storage details were reported. Other details (methods of synthesis, stability, composition etc.) were reported to be on file with the sponsor, but not provided in the study report. The test material was not independently analyzed by the performing laboratory. Comprehensive details of the test diet preparations were provided. Diets were prepared fresh weekly and stored refrigerated in non-plastic containers. They were mixed to homogeneity and stability was tested. Concentrations of the test material in the diets were measured using reverse-phase, high performance liquid chromatography during weeks 1, 13, 26, 52, 78, and 104. Target concentrations in food and calculated average daily doses were provided. Concentration analysis indicated concentrations remained within 92 – 112% of the targets, which was considered by the authors to be acceptable. Individual food intake and body weight data were provided in a dietary study and average daily doses were calculated. The route of exposure was justified by the authors.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for an oncogenicity study and were consistent across groups. Mice were exposed daily, via the diet, for at least 104 weeks.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species was justified by the study authors, but no justification for the strain selected was provided. The number of animals per group was appropriate. Detailed methods were described for all of the endpoints evaluated including the timing of outcome assessment and sample sizes. Leukocyte differential and cellular morphology analysis were performed only for the control and high dose animals at week 78. Significant effects were observed at week 78 in the high dose group (significant decreases in leukocyte, lymphocyte, and/or segmented neutrophil counts); since lower dose groups were not included, this precluded the ability to identify NOEL/LOEL values for these endpoints.
	Metric 9:	Results presentation	High	Individual animal data were provided for all of the endpoints specified. Statistical methods were clearly described and were appropriate for the datasets.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Immune/Hematological-Hematology (RBC, counts of erythrocytes, reticulocytes, platelets, and leukocytes [corrected and non-corrected], MCH, MCHC, MCV, hematocrit, hemoglobin, leukocyte differential (control and high-dose only), myeloid/erythroid ratios at necropsy; organ weights (spleen); Gross necropsy; histopathology (spleen and thymus, lymph nodes).-Neurological/Behavioral-Behavioral-related clinical signs; gross examination of the brain and spinal cord; organ weights (brain with stem); Gross necropsy; histopathology (brain with stem, peripheral nerves, spinal cord)-Reproductive/Developmental-Organ weights (testes with epididymides, uterus); gross necropsy; histopathology (prostate, seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries).-Lung/Respiratory-Respiratory-related clinical signs; Gross examinations of the nasal cavity and paranasal sinuses; lung weights; gross necropsy; histopathology (lung, trachea).-Cardiovascular-Histopathology (heart); gross necropsy; histopathology (heart, aorta).-Gastrointestinal-Gross necropsy; Histopathology (large intestines, stomach, esophagus, salivary glands, small intestines).-Thyroid-Histopathology (thyroid and parathyroid).-Other (please specify below) (Endocrine)-Gross necropsy; Histopathology (adrenal glands, pancreas, pituitary).-Musculoskeletal-Clinical signs (malocclusion); Gross necropsy (skeletal muscle, femur, bone mandibular)-Ocular/Sensory-Clinical observations; Gross necropsy (eye, lacrimal glands)-Skin/Connective Tissue-Skin-related clinical observations; Gross necropsy-Other (please specify below) (Gross necropsy (general)-necropsy of all orifices; carcass; thoracic, abdominal, and pelvic cavities/viscera-Cancer/Carcinogenesis-Microscopic examinations for tumors-Other (please specify below) (Clinical signs (non-target specific)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported, including details of the test substance (DINP, source, purity), animal model (species, strain, sex, age, source, starting body weights), animal husbandry conditions (housing/cage details, animals/cage, food and water availability, room temperature, humidity, light cycle, air changes), the method of administration, and experimental design details. Quantitative results were reported for all of the endpoints described.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated into study groups using a computerized-weight-randomization program. Animals selected for interim sacrifices were selected from animals with the lowest animal identification numbers within each group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified; however, the endpoints were either simple or not subjective (e.g., mortality, organ weight measurements), or blinding is not recommended (initial histopathology), or required in current guidelines for the endpoints (clinical observations and gross necropsy).
Domain 3: Confounding / Variable Control				

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Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Immune/Hematological-Hematology (RBC, counts of erythrocytes, reticulocytes, platelets, and leukocytes [corrected and non-corrected], MCH, MCHC, MCV, hematocrit, hemoglobin, leukocyte differential (control and high-dose only), myeloid/erythroid ratios at necropsy; organ weights (spleen); Gross necropsy; histopathology (spleen and thymus, lymph nodes).-Neurological/Behavioral-Behavioral-related clinical signs; gross examination of the brain and spinal cord; organ weights (brain with stem); Gross necropsy; histopathology (brain with stem, peripheral nerves, spinal cord)-Reproductive/Developmental-Organ weights (testes with epididymides, uterus); gross necropsy; histopathology (prostate, seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries).-Lung/Respiratory-Respiratory-related clinical signs; Gross examinations of the nasal cavity and paranasal sinuses; lung weights; gross necropsy; histopathology (lung, trachea).-Cardiovascular-Histopathology (heart); gross necropsy; histopathology (heart, aorta).-Gastrointestinal-Gross necropsy; Histopathology (large intestines, stomach, esophagus, salivary glands, small intestines).-Thyroid-Histopathology (thyroid and parathyroid).-Other (please specify below) (Endocrine)-Gross necropsy; Histopathology (adrenal glands, pancreas, pituitary).-Musculoskeletal-Clinical signs (malocclusion); Gross necropsy (skeletal muscle, femur, bone mandibular)-Ocular/Sensory-Clinical observations; Gross necropsy (eye, lacrimal glands)-Skin/Connective Tissue-Skin-related clinical observations; Gross necropsy-Other (please specify below) (Gross necropsy (general)-necropsy of all orifices; carcass; thoracic, abdominal, and pelvic cavities/viscera-Cancer/Carcinogenesis-Microscopic examinations for tumors-Other (please specify below) (Clinical signs (non-target specific)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	High	Negative controls were fed basal diets. The negative control responses were appropriate for all of the outcomes specified. Food was analyzed for contamination from other organophosphates, and water was analyzed for contaminates. Animals were housed in metal cages. Some reductions in food consumption was observed, but palatability issues were not mentioned in the study text, and there was no clear relation to dose. No confounding variables that could impact the results of the study were identified.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative results for all endpoints were reported. No animal attrition unrelated to treatment was described. All of the animals were accounted for and sample sizes were clearly reported. There was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test material, source, lot number, purity, and storage details were reported. Other details (stability, composition etc.,) were reported to be on file with the sponsor, but were not provided in the study report. The test material was not independently analyzed by the performing laboratory. Comprehensive details of the test diet preparations were provided. Diets were prepared fresh weekly and stored refrigerated in non-plastic containers. They were mixed to homogeneity and stability was tested. Concentrations of the test material in the diets were measured using reverse-phase, high performance LC during weeks 1, 13, 26, 52, 78, and 104. Target concentrations in food and calculated average daily doses were provided. Individual food intake and body weight data were provided in a dietary study. The route of exposure was justified by the authors.

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Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Mortality-mortality, survival, moribundity-Nutritional/Metabolic-Body weights, body weight gain, mean food consumption.-Immune/Hematological-Hematology (RBC, counts of erythrocytes, reticulocytes, platelets, and leukocytes [corrected and non-corrected], MCH, MCHC, MCV, hematocrit, hemoglobin, leukocyte differential (control and high-dose only), myeloid/erythroid ratios at necropsy; organ weights (spleen); Gross necropsy; histopathology (spleen and thymus, lymph nodes).-Neurological/Behavioral-Behavioral-related clinical signs; gross examination of the brain and spinal cord; organ weights (brain with stem); Gross necropsy; histopathology (brain with stem, peripheral nerves, spinal cord)-Reproductive/Developmental-Organ weights (testes with epididymides, uterus); gross necropsy; histopathology (prostate, seminal vesicles, testes with epididymides, uterus with vagina and cervix, ovaries).-Lung/Respiratory-Respiratory-related clinical signs; Gross examinations of the nasal cavity and paranasal sinuses; lung weights; gross necropsy; histopathology (lung, trachea).-Cardiovascular-Histopathology (heart); gross necropsy; histopathology (heart, aorta).-Gastrointestinal-Gross necropsy; Histopathology (large intestines, stomach, esophagus, salivary glands, small intestines).-Thyroid-Histopathology (thyroid and parathyroid).-Other (please specify below) (Endocrine)-Gross necropsy; Histopathology (adrenal glands, pancreas, pituitary).-Musculoskeletal-Clinical signs (malocclusion); Gross necropsy (skeletal muscle, femur, bone mandibular)-Ocular/Sensory-Clinical observations; Gross necropsy (eye, lacrimal glands)-Skin/Connective Tissue-Skin-related clinical observations; Gross necropsy-Other (please specify below) (Gross necropsy (general)-necropsy of all orifices; carcass; thoracic, abdominal, and pelvic cavities/viscera-Cancer/Carcinogenesis-Microscopic examinations for tumors-Other (please specify below) (Clinical signs (non-target specific)-Non-target specific clinical signs (e.g., missing body part, pale appearance, swollen regions, masses, sores)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain			Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for an oncogenicity study and were consistent across groups. Rats were exposed daily, via the diet, for at least 104 weeks.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The animal species was justified by the study authors, but no justification for the strain selected was provided. The number of animals per group was appropriate. Detailed methods were described for all of the endpoints evaluated including timing and sample sizes. The endpoints were sensitive for the outcomes of interest, or are those generally included in oncogenicity studies (e.g, clinical observations, gross necropsy). All treatment groups were evaluated.
	Metric 9:	Results presentation	High	Individual animal data were provided for all of the endpoints specified. Statistical methods were clearly described and were appropriate for the datasets.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Serum chemistry (albumin, albumin/globulin ratio, AST, ALT, gammy glutamyl transpeptidase, globulin, glucose, total protein, total bilirubin); organ weights; gross necropsy; histopathology; Hepatocellular proliferation rates and biochemical analysis (protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, DNA concentration).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported, including details of the test substance (DINP, source, purity), animal model (species, strain, sex, age, source, starting body weights), animal husbandry conditions (housing/cage details, animals/cage, food and water availability, room temperature, humidity, light cycle, air changes), the method of administration, and experimental design details. Quantitative results were reported for all of the endpoints described.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated into study groups using a computerized-weight-randomization program. Animals selected for interim sacrifices were selected from animals with the lowest animal identification numbers within each group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified; however, the endpoints were either simple or not subjective (e.g., mortality, organ weight measurements), or blinding is not recommended (initial histopathology), or required in current guidelines for the endpoints (clinical observations and gross necropsy).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative controls were fed basal diets. The negative control responses were appropriate for all of the outcomes specified. A positive control group was included for liver proliferation tests for some, but not all time points. The positive control responses were incongruent. The authors attributed this to the "variability or lack of consistency of responses seen among the animals." Some reductions in food consumption were observed, but palatability was not discussed in the study text, and there was no clear relation to dose. No other confounding variables that could impact the results of the study were identified.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative results for all endpoints were reported. No animal attrition unrelated to treatment was described. All of the animals were accounted for and sample sizes were clearly reported. There was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Serum chemistry (albumin, albumin/globulin ratio, AST, ALT, gammy glutamyl transpeptidase, globulin, glucose, total protein, total bilirubin); organ weights; gross necropsy; histopathology; Hepatocellular proliferation rates and biochemical analysis (protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, DNA concentration).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test material, source, lot number, purity, and storage details were reported. Other details (stability, composition etc.,) were reported to be on file with the sponsor, but were not provided in the study report. The test material was not independently analyzed by the performing laboratory. Comprehensive details of the test diet preparations were provided. Diets were prepared fresh weekly and stored refrigerated in non-plastic containers. They were mixed to homogeneity and stability was tested. Concentrations of the test material in the diets were measured using reverse-phase, high performance LC during weeks 1, 13, 26, 52, 78, and 104. Target concentrations in food and calculated average daily doses were provided. Individual food intake and body weight data were provided in a dietary study. The route of exposure was justified by the authors.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for an oncogenicity study and were consistent across groups. Rats were exposed daily, via the diet, for at least 104 weeks.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species was justified by the study authors, but no justification for the strain selected was provided. The sample sizes for the liver cell proliferation and biochemical analyses were small (n = 5); in several cases, due to the presence of leukemia, these animals had to be eliminated from the assessment. Only controls and animals from the two highest doses were included in the 79-week interim sacrifice. Significant effects were observed for some endpoints (e.g., liver and kidney weights). Since lower dose groups were not included, this precluded the ability to identify NOEL/LOEL values for these endpoints.
	Metric 9:	Results presentation	High	Individual animal data were provided for all of the endpoints specified. Statistical methods were clearly described and were appropriate for the datasets.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Renal/Kidney-Serum chemistry (calcium, chloride, inorganic phosphorus, potassium, urea nitrogen, creatinine); urinalysis (volume, osmolality, electrolytes, creatinine, creatinine clearance, appearance, bilirubin, glucose, ketones, occult blood, pH, specific gravity, urobilinogen, microscopic examination of sediment); organ weights; gross necropsy; histopathology (kidney, urinary bladder).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported, including details of the test substance (DINP, source, purity), animal model (species, strain, sex, age, source, starting body weights), animal husbandry conditions (housing/cage details, animals/cage, food and water availability, room temperature, humidity, light cycle, air changes), the method of administration, and experimental design details. Quantitative results were reported for all of the endpoints described.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated into study groups using a computerized-weight-randomization program. Animals selected for interim sacrifices were selected from animals with the lowest animal identification numbers within each group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified; however, the endpoints were either simple or not subjective (e.g., mortality, organ weight measurements), or blinding is not recommended (initial histopathology), or required in current guidelines for the endpoints (clinical observations and gross necropsy).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	Negative controls were fed basal diets. The negative control responses were appropriate for all of the outcomes specified. Food was analyzed for contamination from other organophosphates, and water was analyzed for contaminates. Animals were housed in metal cages. Some reductions in food consumption was observed, but palatability issues were not mentioned in the study text, and there was no clear relation to dose. No confounding variables that could impact the results of the study were identified.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative results for all endpoints were reported. No animal attrition unrelated to treatment was described. All of the animals were accounted for and sample sizes were clearly reported. There was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Covance Labs, (1998). Support: Oncogenicity study in rats with di(isononyl) phthalate including ancillary hepatocellular proliferation & biochemical analyses with cover.
Health Outcome(s) and Reported Health Effect(s):	Renal/Kidney-Serum chemistry (calcium, chloride, inorganic phosphorus, potassium, urea nitrogen, creatinine); urinalysis (volume, osmolality, electrolytes, creatinine, creatinine clearance, appearance, bilirubin, glucose, ketones, occult blood, pH, specific gravity, urobilinogen, microscopic examination of sediment); organ weights; gross necropsy; histopathology (kidney, urinary bladder).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-104-week(s)
Species:	Rat-Other (CDF (F-344) CrlBR)-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680087

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test material, source, lot number, purity, and storage details were reported. Other details (stability, composition etc.,) were reported to be on file with the sponsor, but were not provided in the study report. The test material was not independently analyzed by the performing laboratory. Comprehensive details of the test diet preparations were provided. Diets were prepared fresh weekly and stored refrigerated in non-plastic containers. They were mixed to homogeneity and stability was tested. Concentrations of the test material in the diets were measured using reverse-phase, high performance LC during weeks 1, 13, 26, 52, 78, and 104. Target concentrations in food and calculated average daily doses were provided. Individual food intake and body weight data were provided in a dietary study. The route of exposure was justified by the authors.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for an oncogenicity study and were consistent across groups. Rats were exposed daily, via the diet, for at least 104 weeks.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The animal species was justified by the study authors, but no justification for the strain selected was provided. Only controls and animals from the two highest doses were included in the 79-week interim sacrifice. Significant effects were observed for some endpoints (e.g., liver and kidney weights). Since lower dose groups were not included, this precluded the ability to identify NOEL/LOEL values for these endpoints. The sample sizes were adequate for the endpoints specified.
	Metric 9:	Results presentation	High	Individual animal data were provided for all of the endpoints specified. Statistical methods were clearly described and were appropriate for the datasets.

Additional Comments: None

Overall Quality Determination High

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) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784622

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 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 / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included. Low levels of phthalate metabolites were detected 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 drinking 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. Drinking 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 exposures. 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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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) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784622

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	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 Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma-Aldrich) was reported. The purity was reported to be ≥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 Measures and Results Display				
	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.

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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) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784622

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

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) and Reported Health Effect(s):	Reproductive/Developmental-Estrous cycle; Fertility indices (mating index, gestational index, pregnancy, birth rate, dystocia and fertility index)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784622

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	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 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 / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included. Low levels of phthalate metabolites were detected 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 drinking 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. Drinking 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 exposures. 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				

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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) and Reported Health Effect(s):	Reproductive/Developmental-Estrous cycle; Fertility indices (mating index, gestational index, pregnancy, birth rate, dystocia and fertility index)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784622

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 Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma-Aldrich) was reported. The purity was reported to be ≥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 Measures and Results 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 guidelines (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.

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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) and Reported Health Effect(s):	Reproductive/Developmental-Estrous cycle; Fertility indices (mating index, gestational index, pregnancy, birth rate, dystocia and fertility index)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-11-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784622

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 Quality Determination Low

Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-6-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784618

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified as di(2-ethylhexyl)phthalate. No CASRN was provided. 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. Husbandry conditions (temperature, humidity, and light cycle) were not reported. Animals were housed 3/cage. Feed and water were available ad libitum. Dose levels (ppm), duration, 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 concentrations were reported; however, actual doses were not. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and 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 / 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 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				

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Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-6-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784618

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 Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	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.
	Metric 7:	Exposure timing, frequency, and duration	High	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 Measures and Results 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 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 ± 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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Study Citation:	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.
Health Outcome(s) and Reported Health Effect(s):	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
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-6-month(s)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784618

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

Study Citation:	Bhurke, A., Davila, J., Flaws, J. A., Bagchi, M. K., Bagchi, I. C. (2023). Exposure to di-isononyl phthalate during early pregnancy disrupts decidual angiogenesis and placental development in mice. Reproductive Toxicology 120:108446.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Gestation length, litter size, pup weight on PND 1, and sex ratio. Maternal serum estrogen and progesterone levels, number of implantation sites, gross uterine pathology, fetal and placental weight, histopathology and immunohistochemistry (implantation chambers and placenta), measurement of total area of the placenta, junctional zone and labyrinth, ratio of labyrinth to junctional zone, and relative mRNA expression in placenta, decidua and embryo tissues for genes involved in decidualization process, angiogenic regulators, and placental cell types.
Duration and Exposure Route:	Other (specify) (Orally piped directly into the mouth)-Duration: Reproductive/Developmental-1-F0 - gestation (GD 1-7)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784571

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	Test substance identity was confirmed via name. Purity and commercial source were provided along with catalog number. Test animal species, sex, strain, starting age and commercial source were reported. Starting body weights were not reported. Parity was not reported. Animal husbandry conditions such as number of animals/cage, food and water availability, temperature and light/dark cycle were reported. Humidity was not reported. Exposure administration methods and endpoint assessment methodology were reported. Missing information is not expected to significantly impact the quality of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The authors state that animals were randomly allocated into groups, but did not state the method of randomization.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods to reduce observational bias were not described, but all endpoints were objective in nature or included initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	An appropriate negative control was included, and responses were appropriate. A positive control is not required for this study type. The authors did confirm that the corn oil vehicle was stripped of tocopherol, which could be a confounder in this study. The dosing volume delivered varied from 28 to 41 uL (based on daily body weight measurements); it is unclear if differences existed between the control and exposed groups. Body weights and food intake were not reported. The study did not report taking measures to minimize the exposure to other plasticizers. The type of cage animals were housed in or food and water dispensing containers were not reported. 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.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	All animals are accounted for in the results and the authors did not describe any maternal health outcomes that could be unrelated to exposure. Not all outcomes described in the methods were reported or shown (e.g. serum hormone levels, placental weight).

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Study Citation:	Bhurke, A., Davila, J., Flaws, J. A., Bagchi, M. K., Bagchi, I. C. (2023). Exposure to di-isononyl phthalate during early pregnancy disrupts decidual angiogenesis and placental development in mice. Reproductive Toxicology 120:108446.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Gestation length, litter size, pup weight on PND 1, and sex ratio. Maternal serum estrogen and progesterone levels, number of implantation sites, gross uterine pathology, fetal and placental weight, histopathology and immunohistochemistry (implantation chambers and placenta), measurement of total area of the placenta, junctional zone and labyrinth, ratio of labyrinth to junctional zone, and relative mRNA expression in placenta, decidua and embryo tissues for genes involved in decidualization process, angiogenic regulators, and placental cell types.
Duration and Exposure Route:	Other (specify) (Orally piped directly into the mouth)-Duration: Reproductive/Developmental-1-F0 - gestation (GD 1-7)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11784571

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Reported test substance purity is ideal, but the authors did not perform their own analytical verification of test substance purity. Test substance storage conditions were not described, though due to the high stability of the test substance at room temperature, this deficiency is unlikely to strongly impact the results. Test substance preparation and administration methods were described. The vehicle control or test substance were orally piped directly into the mouth of mice. The study does not report if they observed the mice to ensure they swallowed the administered dose. Delivered volume was reported as between 28 and 41 uL and was adjusted based on daily body weights to deliver 20 ug/kg/day. Target administered dose levels were reported in ug/kg/day, but the authors did not state whether these dose levels were analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency and duration are consistent between groups and are sensitive to determine the outcomes of interest. The study authors note GD 1-7 “encompasses both embryo attachment to the uterine epithelium and decidualization. . . ”.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The species, strain and sample size and endpoint evaluation methods were appropriate to measure endpoints of interest. The study’s aim was to study a time course of events that may be responsible for decreased fetal survival, therefore sacrificing animals at different timepoints was appropriate. The methods were not reported in detail, and it is unclear if all tests were performed on all groups of animals (e.g. serum hormones or placental measurement). Only one dose of the test substance was used, which is a minor limitation given that a dose below the LOAEL was not included, reducing the ability to use this study to determine dose-response of the outcome. The dose was justified as an ”environmentally relevant dose”.
	Metric 9:	Results presentation	Low	Quantitative or qualitative data were presented for most endpoints, however there were several endpoints where data were not reported. Statistical methods were described. The study authors used the pup, rather than the litter as the unit for statistical analysis. Individual animal data were not provided. Analyzing offspring data in this manner is not recommended and can overestimate the statistical significance (Dishaw et al. 2020).

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Biomedical,, Exxon (1996). Reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain, food consumption-Mortality-Survival-Reproductive/Developmental-Organ weights (left and right testis and epididymis, prostate, seminal vesicles, left and right ovaries), and gross observations. Indices for mating, fertility, fecundity, offspring survival from birth to weaning, lactation, gestation and live births, number of days of gestation, litter size, number of live and dead offspring, % of live males and females, offspring body weights,-Hepatic/Liver-Absolute and relative liver weights, gross observation-Renal/Kidney-Absolute and relative kidney weight; gross observations-Other (please specify below) (Clinical signs)-Clinical signs of toxicity.-Other (please specify below) (Gross necropsy)-Gross necropsy-Skin/Connective Tissue-Gross observation-Other (please specify below) (Endocrine)-Gross observation of pituitary-Ocular/Sensory-Gross observation of eye-Lung/Respiratory-Gross observation of lung-Other (please specify below) (Dental)-Gross observation on teeth
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating-F0 - gestation (GD 0-21)-F0- lactation (PND 0-21)-F0- pre-mating (10 weeks)-F0- mating
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987588 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	Test substance is identified definitively by name and CASRN and the source and purity are reported. Test animal characteristics (such as species, strain, sex, source, and parity) are reported. Test animal starting age is not reported, and starting body weights are reported in body weight data in the results, but not in the methods. Animal housing conditions (such as number of animals per cage, food and water availability, light/dark cycle, temperature and humidity) are all reported. Experimental methods and exposure details are reported in adequate detail.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to groups on the basis of body weight normalization using a computer-generated randomization procedure.
	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, food intake, measurable endpoint) or clinical signs.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Due to the dietary and ad libitum nature of the exposure, food palatability is a significant concern. The authors do report adequate information to determine confounding, but significant differences in food consumption at higher doses do imply that palatability may be a significant problem. Animal husbandry conditions were consistent across groups. An appropriate negative control is reported, and there are no concerns with control response. Analysis of food, water and bedding found there were no know contaminants "believed to have been present at levels that may have interfered with this study".
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Biomedical,, Exxon (1996). Reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain, food consumption-Mortality-Survival-Reproductive/Developmental-Organ weights (left and right testis and epididymis, prostate, seminal vesicles, left and right ovaries), and gross observations. Indices for mating, fertility, fecundity, offspring survival from birth to weaning, lactation, gestation and live births, number of days of gestation, litter size, number of live and dead offspring, % of live males and females, offspring body weights,-Hepatic/Liver-Absolute and relative liver weights, gross observation-Renal/Kidney-Absolute and relative kidney weight; gross observations-Other (please specify below) (Clinical signs)-Clinical signs of toxicity.-Other (please specify below) (Gross necropsy)-Gross necropsy-Skin/Connective Tissue-Gross observation-Other (please specify below) (Endocrine)-Gross observation of pituitary-Ocular/Sensory-Gross observation of eye-Lung/Respiratory-Gross observation of lung-Other (please specify below) (Dental)-Gross observation on teeth
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating-F0 - gestation (GD 0-21)-F0- lactation (PND 0-21)-F0- premating (10 weeks)-F0- mating
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987588 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	A few unscheduled deaths are reported by the authors, but they are expected from the longer length of this type of study and there were not enough unscheduled deaths to significantly influence the results and the authors specified which from which groups the deaths occurred. All prespecified outcomes are reported in the results and there are no problems with omission bias.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The authors indicate that test substance purity was analytically confirmed and did not contain any contaminants. Regarding test substance preparation, the authors also conducted tests ahead of time to ensure that the test substance was stable in feed for at least 2 weeks, and prepared diets biweekly to ensure there were no issues with exposure administration from test substance stability. Storage conditions were not described, but the test substance is generally stable at room temperature. There are minor uncertainties with administered dose derivation, with nominal doses reported in % in feed, and administered doses reported in mg/kg/day as an uncertain average due to variance in food consumption and body weights.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency and duration of the exposure is sensitive for the endpoints of interest and is consistent between groups.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The chosen species and strain and sample size are appropriate for repro/dev studies. There are no concerns regarding the timing of endpoint assessment. The outcome methodology was sensitive to detect effects of interest. Dose and concentration spacing is adequate, but an additional lower dose would need to be included in order to derive a study-wide NOAEL.
	Metric 9:	Results presentation	High	Statistical methods are described in detail. The litter was not used as the unit of sampling for repro/dev outcomes, but a mixed model that nested litters within dams and used litter size as a covariate was used, so there are likely no concerns regarding statistical analysis. Results presentation is complete with quantitative reporting of all significant outcomes and individual animal data is provided in appendices for outcomes that are not included in figures (such as clinical signs and gross necropsy data). Negative outcomes are described qualitatively in the text, with supporting data in individual animal data in the appendices.

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Study Citation:	Biomedical,, Exxon (1996). Reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain, food consumption-Mortality-Survival-Reproductive/Developmental-Organ weights (left and right testis and epididymis, prostate, seminal vesicles, left and right ovaries), and gross observations. Indices for mating, fertility, fecundity, offspring survival from birth to weaning, lactation, gestation and live births, number of days of gestation, litter size, number of live and dead offspring, % of live males and females, offspring body weights,-Hepatic/Liver-Absolute and relative liver weights, gross observation-Renal/Kidney-Absolute and relative kidney weight; gross observations-Other (please specify below) (Clinical signs)-Clinical signs of toxicity.-Other (please specify below) (Gross necropsy)-Gross necropsy-Skin/Connective Tissue-Gross observation-Other (please specify below) (Endocrine)-Gross observation of pituitary-Ocular/Sensory-Gross observation of eye-Lung/Respiratory-Gross observation of lung-Other (please specify below) (Dental)-Gross observation on teeth
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating-F0 - gestation (GD 0-21)-F0- lactation (PND 0-21)-F0- premating (10 weeks)-F0- mating
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987588 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

Study Citation:	Biomedical,, Exxon (1996). Two generation reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) [unpublished] (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Mortality-Mortality-Other (please specify below) (Clinical signs)-General appearance/non-organ specific clinical signs, overt signs of toxicity-Hepatic/Liver-Gross necropsy of liver, liver weight, histopathology of liver-Renal/Kidney-Gross necropsy of kidney and urinary bladder, kidney weight, histopathology of kidney and urinary bladder-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Gross necropsy and histopathology of pituitary gland-Reproductive/Developmental-Parental reproductive endpoints: Male mating, male and female fertility, female fecundity, gestational index, and gestation length; gross necropsy and organ weights of testes, epididymides, prostate, seminal vesicles, and ovaries; histopathology of vagina, uterus (with cervix), ovaries, mammary gland (females only), coagulating gland, testes, epididymides, seminal vesicles, and prostate. Developmental endpoints: litter size, live offspring per litter, percentage of male and female offspring per litter; offspring survival, viability at weaning, body weights, body weight gain during lactation; pup clinical signs, and gross necropsy of pups at termination.-Lung/Respiratory-Clinical signs (rales), histopathology of lungs-Gastrointestinal-Gross necropsy of cecum, colon, ileum, large intestines, rectum, and stomach; histopathology of cecum, colon, rectum, and stomach-Immune/Hematological-Gross necropsy of thymus, spleen, and lymph nodes; histopathology of spleen and lymphoreticular system-Skin/Connective Tissue-Gross necropsy of skin/subcutis
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (3 weeks)-F0 - gestation (22 days)-F0- lactation (21 days)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)-F1 - gestation (22 days)-F1- lactation (21 days)-F0- premating (10 weeks)-F0- mating (3 weeks)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987589 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported including details of the test substance (name, source, purity), test animal characteristics (species, strain, sex, age, starting body weights, and parity), husbandry conditions (temperature, humidity, light cycle, food and water availability, animals per cage), and information about the experimental design and endpoint evaluation methods. Quantitative results were reported for all of the endpoints identified.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Parental animals (P1) were allocated randomly to groups by a computer-generated randomization procedure based on body weight. Selection of offspring at PND 21 for sacrifice and examination of internal abnormalities (10/sex/group) and selection of offspring (maximum of 2/sex/litter) for the F1 generation was done randomly.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were not reported; however, this is not expected to significantly impact the study because the endpoints evaluated are not subjective or blinding is otherwise not required (e.g., clinical signs, initial histopathology).
Domain 3: Confounding / Variable Control				

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Study Citation:	Biomedical,, Exxon (1996). Two generation reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) [unpublished] (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Mortality-Mortality-Other (please specify below) (Clinical signs)-General appearance/non-organ specific clinical signs, overt signs of toxicity-Hepatic/Liver-Gross necropsy of liver, liver weight, histopathology of liver-Renal/Kidney-Gross necropsy of kidney and urinary bladder, kidney weight, histopathology of kidney and urinary bladder-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Gross necropsy and histopathology of pituitary gland-Reproductive/Developmental-Parental reproductive endpoints: Male mating, male and female fertility, female fecundity, gestational index, and gestation length; gross necropsy and organ weights of testes, epididymides, prostate, seminal vesicles, and ovaries; histopathology of vagina, uterus (with cervix), ovaries, mammary gland (females only), coagulating gland, testes, epididymides, seminal vesicles, and prostate. Developmental endpoints: litter size, live offspring per litter, percentage of male and female offspring per litter; offspring survival, viability at weaning, body weights, body weight gain during lactation; pup clinical signs, and gross necropsy of pups at termination.-Lung/Respiratory-Clinical signs (rales), histopathology of lungs-Gastrointestinal-Gross necropsy of cecum, colon, ileum, large intestines, rectum, and stomach; histopathology of cecum, colon, rectum, and stomach-Immune/Hematological-Gross necropsy of thymus, spleen, and lymph nodes; histopathology of spleen and lymphoreticular system-Skin/Connective Tissue-Gross necropsy of skin/subcutis
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (3 weeks)-F0 - gestation (22 days)-F0- lactation (21 days)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)-F1 - gestation (22 days)-F1- lactation (21 days)-F0- premating (10 weeks)-F0- mating (3 weeks)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987589 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	High	The study included an appropriate basal diet control. The control responses were appropriate for all of the outcomes specified. Animals were housed in stainless steel cages. Bedding, food, and water were analyzed for contaminants. It is unclear if they were analyzed for other organophosphates. The authors reported that there were no known contaminants that could have interfered with the study. Animal husbandry conditions were consistent across groups. It was noted that half of the animals were infected with pinworms (observed in all dose groups). These are non-pathogenic and no downstream associations with observed health effects were reported.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals are accounted for in the presented results and there were no unexplained omissions or attrition. Results were presented for all prespecified outcomes.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance supplier was specified. The test material stability, strength, purity and composition were determined by the test laboratory but the analytical data were not provided. The purity was assumed as 100% for dosing. Homogeneity, stability (during 14 days), and achieved concentrations of the test substance in feed were determined. Measured concentrations remained within 15% of nominal. Diets were prepared fresh weekly. Food consumption was measured for both males and females and actual doses (mg/kg/day) were calculated.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure appear to be appropriate for the study type and the outcomes evaluated.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Biomedical,, Exxon (1996). Two generation reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) [unpublished] (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Mortality-Mortality-Other (please specify below) (Clinical signs)-General appearance/non-organ specific clinical signs, overt signs of toxicity-Hepatic/Liver-Gross necropsy of liver, liver weight, histopathology of liver-Renal/Kidney-Gross necropsy of kidney and urinary bladder, kidney weight, histopathology of kidney and urinary bladder-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Gross necropsy and histopathology of pituitary gland-Reproductive/Developmental-Parental reproductive endpoints: Male mating, male and female fertility, female fecundity, gestational index, and gestation length; gross necropsy and organ weights of testes, epididymides, prostate, seminal vesicles, and ovaries; histopathology of vagina, uterus (with cervix), ovaries, mammary gland (females only), coagulating gland, testes, epididymides, seminal vesicles, and prostate. Developmental endpoints: litter size, live offspring per litter, percentage of male and female offspring per litter; offspring survival, viability at weaning, body weights, body weight gain during lactation; pup clinical signs, and gross necropsy of pups at termination.-Lung/Respiratory-Clinical signs (rales), histopathology of lungs-Gastrointestinal-Gross necropsy of cecum, colon, ileum, large intestines, rectum, and stomach; histopathology of cecum, colon, rectum, and stomach-Immune/Hematological-Gross necropsy of thymus, spleen, and lymph nodes; histopathology of spleen and lymphoreticular system-Skin/Connective Tissue-Gross necropsy of skin/subcutis
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (3 weeks)-F0 - gestation (22 days)-F0- lactation (21 days)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)-F1 - gestation (22 days)-F1- lactation (21 days)-F0- premating (10 weeks)-F0- mating (3 weeks)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987589 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	The procedures were sensitive and specific for evaluating the outcomes. The dose spacing was justified and appropriate. However, the lowest dose (LOAEL for systemic and developmental effects) induced effects (i.e., no NOAEL identified in the study). Doses were selected based on the results of a one-generation range-finding oral toxicity study in rats with dose levels of 0, 0.5, 1, and 1.5% in which effects on parental animals and offspring were observed at 0.5% and higher. The animal model used in the study and the group sizes were appropriate for the study type and study design. The outcomes evaluated were assessed consistently across groups during the study. Animals were observed for overt signs of toxicity daily and clinical examinations were performed in parental animals weekly during premating and mating in males and females, and during gestation in females, and then on PND 0, 4, 7, 10, 14, and 21 in females. Some organ examinations were limited to histopathology (e.g., pituitary) or organ weight (e.g., brain weight) and some reproductive organ function measures (e.g., sperm evaluations; estrous cyclicity) and developmental measures (litter weights) were not conducted in this study; however, this is not expected to substantially impact the results.
	Metric 9:	Results presentation	High	The results presentation is appropriate for the study type and endpoints evaluated. All deaths were clearly reported including the times and causes of death. Mean values for each group (e.g., adult body weight; adult food consumption; pup body weight) are provided with variance and are reported by dose group. Individual data are reported in appendices. The statistical methods utilized to assess the results were appropriate. Some of the data presented in the results section in HERO ID 1987589 for developmental toxicity are averaged across pups in a treatment group rather than litter (e.g., pup body weight; Table 19 [F1] and Table 38 [F2] from which the summary results averaged across all pups are presented in the main body of the report on p. 36 for F1, and p. 46 for F2. Additionally, the body weight data averaged across pups rather than litters are also presented in summary tables in HERO ID 680202. However, this is not expected to affect the data quality because individual data were provided which would allow an independent analysis of the results and statistical analyses described in HERO ID 680202 indicate that litters were utilized in the statistical analyses.

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Study Citation:	Biomedical,, Exxon (1996). Two generation reproduction toxicity study in rats with diisononyl phthalate (DINP; MRD-92-455) [unpublished] (sanitized).
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Mortality-Mortality-Other (please specify below) (Clinical signs)-General appearance/non-organ specific clinical signs, overt signs of toxicity-Hepatic/Liver-Gross necropsy of liver, liver weight, histopathology of liver-Renal/Kidney-Gross necropsy of kidney and urinary bladder, kidney weight, histopathology of kidney and urinary bladder-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Gross necropsy and histopathology of pituitary gland-Reproductive/Developmental-Parental reproductive endpoints: Male mating, male and female fertility, female fecundity, gestational index, and gestation length; gross necropsy and organ weights of testes, epididymides, prostate, seminal vesicles, and ovaries; histopathology of vagina, uterus (with cervix), ovaries, mammary gland (females only), coagulating gland, testes, epididymides, seminal vesicles, and prostate. Developmental endpoints: litter size, live offspring per litter, percentage of male and female offspring per litter; offspring survival, viability at weaning, body weights, body weight gain during lactation; pup clinical signs, and gross necropsy of pups at termination.-Lung/Respiratory-Clinical signs (rales), histopathology of lungs-Gastrointestinal-Gross necropsy of cecum, colon, ileum, large intestines, rectum, and stomach; histopathology of cecum, colon, rectum, and stomach-Immune/Hematological-Gross necropsy of thymus, spleen, and lymph nodes; histopathology of spleen and lymphoreticular system-Skin/Connective Tissue-Gross necropsy of skin/subcutis
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (3 weeks)-F0 - gestation (22 days)-F0- lactation (21 days)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)-F1 - gestation (22 days)-F1- lactation (21 days)-F0- premating (10 weeks)-F0- mating (3 weeks)-F1- premating (PND 21, 11 weeks before mating)-F1- mating (3 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1987589 Linked HERO ID(s): 1987588, 680202, 1987589

Domain	Metric	Rating	Comments

Additional Comments: None

Overall Quality Determination High

Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testes histopathology, ex vivo testosterone production, serum and testes testosterone content, histopathology of reproductive organs and thyroids, gestation length, litter size, number of fetuses, number of implantations, number of live fetuses, percent post-implantation loss, litter weights, sex ratio, survival, anogenital distance, pup weights, presence/retention of nipples/areolas, age of sexual maturation, inhibin B analysis, body weights at post-natal day 90, penile malformation, testicular descent, organ weight (liver, kidney, adrenal, thyroid, testes, epididymis, seminal vesicle, ventral prostate, levator ani/bulbocavernosus muscle, bulborethral gland, ovaries, uterus), histopathology (testes, epididymides), sperm parameters, including motility, first day of estrous, motor activity and habituation capability (including Morris maze learning and memory)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical information is present. Authors provided the species (rat), test article identity (chemical name and CAS No provided), dose levels (given in mg DINP/kg body weight per day), exposure duration (daily from gestation day 7 to post-natal day 17), exposure route (oral), and results for at least one endpoint (data presented in multiple graphs and tables, as well as in the text). Authors also provided all important information regarding test animals. Specifically, the test animals were Wistar time mated female rats weighing approximately 200 grams from Taconic M&B in Denmark. They were housed in pairs in plastic cages in controlled environmental conditions (12-hour light–dark cycles with light starting at 9 p.m., temperature 22 $\pm$ 2 °C, humidity 55% $\pm$ 5%, ventilation 10 air changes per hour). Food and tap water were provided ad libitum. Mothers as well as male and female offspring evaluated. Important information regarding exposure methods (test substance acquired from Aldrich, 99% purity, oral gavage in corn oil vehicle), experimental design (daily exposures, 16 dams per study group with age relative to the time of conception provided for all animals), and endpoint evaluation methods (some of these methods were elaborated in cited references).
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly distributed into groups with similar body weight distributions. The randomization method was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias were reported for certain endpoints. The histology endpoints (targeted histopathology conducted specifically on male reproductive organs) were evaluated by a blinded observer, as were the anogenital distance and nipple retention endpoints. Evaluators for other endpoints were not blinded, but the potential for bias was mitigated because the outcomes of these endpoints were based on the use of a computer driven system (sperm quality motility), laboratory kits (testosterone levels, testosterone production), or were simple objective measures (maternal pup retrieval, age at sexual maturation, organ weight).
Domain 3: Confounding / Variable Control				

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Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testes histopathology, ex vivo testosterone production, serum and testes testosterone content, histopathology of reproductive organs and thyroids, gestation length, litter size, number of fetuses, number of implantations, number of live fetuses, percent post-implantation loss, litter weights, sex ratio, survival, anogenital distance, pup weights, presence/retention of nipples/areolas, age of sexual maturation, inhibin B analysis, body weights at post-natal day 90, penile malformation, testicular descent, organ weight (liver, kidney, adrenal, thyroid, testes, epididymis, seminal vesicle, ventral prostate, levator ani/bulbocavernosus muscle, bulborethral gland, ovaries, uterus), histopathology (testes, epididymides), sperm parameters, including motility, first day of estrous, motor activity and habituation capability (including Morris maze learning and memory)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	Medium	Food and water intake were not monitored in this oral gavage study. There were no significant differences in maternal body weights that could confound exposure. An appropriate negative control condition was utilized (the corn oil vehicle). The negative control response was generally acceptable. However, some negative control responses showed a large degree of variability (testicular testosterone production) or high background incidence (histological changes in the adult prostate and testes). Despite the purpose of the paper investigating an endocrine disrupting chemical, the authors do not detail any methods to minimize outside exposure to other plasticizers. Exposure to other plasticizers could impact negative control response and confound the study. There was no positive control condition in this study. It is not clear why the authors reported only 8-9 litters/group when there are 16 dams/group.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Results were reported for all prespecified outcomes, exposure groups and evaluation time points. For endpoints in which there was no difference between experimental and control groups, the results were described in the text with no data reported. In the 900 mg/kg-day exposure group, the testis was damaged and histology could not be performed (though the authors were able to confirm the presence of multinucleated gonocytes).
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	Test chemical identity, test substance source, and test substance purity were all reported. The purity reported appears to be provided by the vender. The preparation and storage of the chemical was not adequately reported; the authors do state that they use corn oil as a vehicle but no additional information on preparation is given. Gavage volume was not provided. Nominal dose concentration was reported but the authors do not use any analytical methods to confirm the doses. The minor uncertainties about precision of dose levels do not impact interpretation of results.

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Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testes histopathology, ex vivo testosterone production, serum and testes testosterone content, histopathology of reproductive organs and thyroids, gestation length, litter size, number of fetuses, number of implantations, number of live fetuses, percent post-implantation loss, litter weights, sex ratio, survival, anogenital distance, pup weights, presence/retention of nipples/areolas, age of sexual maturation, inhibin B analysis, body weights at post-natal day 90, penile malformation, testicular descent, organ weight (liver, kidney, adrenal, thyroid, testes, epididymis, seminal vesicle, ventral prostate, levator ani/bulbocavernosus muscle, bulborethral gland, ovaries, uterus), histopathology (testes, epididymides), sperm parameters, including motility, first day of estrous, motor activity and habituation capability (including Morris maze learning and memory)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	The authors did not attempt to justify the exposure frequency and timing that they used. Exposure timing, duration, and frequency appear to include the critical sensitivity period for certain endpoints that the authors were investigating. Numerous developmental and reproductive endpoints (including nipple retention, anogenital distance, sperm motility, sperm count) were significantly different from the negative control group following exposure, implying that the experimental design is sufficient to detect exposure related differences in these domains. However, given that the exposure period started on day 7, it may not have been appropriate for measuring effects on organogenesis (guidelines recommend starting exposure on gestational day 5). Minimal details regarding exposure administration are reported (time of day of administration).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The number of exposure groups and dose range/spacing is appropriate for the purposes of this study and based on previously published literature. For the hormone analysis endpoints, the authors do specify that they only examined one section from each of 5 to 7 testes representing 3 to 4 litters. This sample size is acceptable but less-than-ideal. The outcome assessment methodology (evaluating fetal and adult offspring testosterone production, reproductive histology, nipple retention, and sperm count) was appropriate given the author's interest in the anti-androgenic effects of DINP. The outcome assessments were consistently applied across experimental groups.
	Metric 9:	Results presentation	High	Statistical methods used were clearly described and appropriate for the study. To account for litter effects, when more than one pup from each litter was examined, statistical analyses was adjusted using litter as an independent random and nested factor in ANOVA. Quantitative results were reported for each outcome where there was a significant difference between control and treated groups. These results were reported as incidence data or as mean +/- standard deviation or standard error. For endpoints where there were no significant differences between control and treated groups, qualitative results were reported. The results of all endpoints were reported.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Maternal pup retrieval test
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical information is present. Authors provided the species (rat), test article identity (chemical name and CAS No provided), dose levels (given in mg DINP/kg body weight per day), exposure duration (daily from gestation day 7 to post-natal day 17), exposure route (oral), and results for at least one endpoint (data presented in multiple graphs and tables, as well as in the text). Authors also provided all important information regarding test animals. Specifically, the test animals were Wistar time mated female rats weighing approximately 200 grams from Taconic M&B in Denmark. They were housed in pairs in plastic cages in controlled environmental conditions (12-hour light–dark cycles with light starting at 9 p.m., temperature 22 $\pm$ 2 °C, humidity 55% $\pm$ 5%, ventilation 10 air changes per hour). Food and tap water were provided ad libitum. Mothers as well as male and female offspring evaluated. Important information regarding exposure methods (test substance acquired from Aldrich, 99% purity, oral gavage in corn oil vehicle), experimental design (daily exposures, 16 dams per study group with age relative to the time of conception provided for all animals), and endpoint evaluation methods (some of these methods were elaborated in cited references).
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly distributed into groups with similar body weight distributions. The randomization method was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Low	There is no indication that the evaluators of behavioral endpoints were blinded. The potential impact of this lack of blinding is significant.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Food and water intake were not monitored in this oral gavage study. There were no significant differences in maternal body weights that could confound exposure. An appropriate negative control condition was utilized (the corn oil vehicle). The negative control response was not reported for behavioral endpoints. Despite the purpose of the paper investigating an endocrine disrupting chemical, the authors do not detail any methods to minimize outside exposure to other plasticizers. Exposure to other plasticizers could impact negative control response and confound the study. There was no positive control condition in this study. It is not clear why the authors reported only 8-9 litters/group when there are 16 dams/group.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Maternal pup retrieval test
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Results for behavioral endpoints were reported in the body of the text. No data was reported, but the authors clearly report that there was no difference between treatment and control conditions.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	Test chemical identity, test substance source, and test substance purity were all reported. The purity reported appears to be provided by the vender. The preparation and storage of the chemical was not adequately reported; the authors do state that they use corn oil as a vehicle but no additional information on preparation is given. Gavage volume was not provided. Nominal dose concentration was reported but the authors do not use any analytical methods to confirm the doses. The minor uncertainties about precision of dose levels do not impact interpretation of results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure frequency, duration and timing were not justified by the authors, but seemed to be appropriate for this outcome of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The number of exposure groups and dose range/spacing is appropriate for the purposes of this study and based on previously published literature. The sample size is appropriate. The outcome assessment methodology (pup retrieval time) was appropriate given the author's interest in the masculinization of behavior following DINP exposure. The outcome assessments were consistently applied across experimental groups.
	Metric 9:	Results presentation	Medium	Statistical methods used were clearly described and appropriate for the study. Data for behavioral endpoints are reported qualitatively (no quantitative data was reported); the authors state in the text that there was no difference between treated and control conditions.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Dam body weights and weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical information is present. Authors provided the species (rat), test article identity (chemical name and CAS No provided), dose levels (given in mg DINP/kg body weight per day), exposure duration (daily from gestation day 7 to post-natal day 17), exposure route (oral), and results for at least one endpoint (data presented in multiple graphs and tables, as well as in the text). Authors also provided all important information regarding test animals. Specifically, the test animals were Wistar time mated female rats weighing approximately 200 grams from Taconic M&B in Denmark. They were housed in pairs in plastic cages in controlled environmental conditions (12-hour light–dark cycles with light starting at 9 p.m., temperature 22 $\pm$ 2 °C, humidity 55% $\pm$ 5%, ventilation 10 air changes per hour). Food and tap water were provided ad libitum. Mothers as well as male and female offspring evaluated. Important information regarding exposure methods (test substance acquired from Aldrich, 99% purity, oral gavage in corn oil vehicle), experimental design (daily exposures, 16 dams per study group with age relative to the time of conception provided for all animals), and endpoint evaluation methods (some of these methods were elaborated in cited references).
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly distributed into groups with similar body weight distributions. The randomization method was not specified.
	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 simple objective measures (maternal weight, maternal weight gain).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Food and water intake were not monitored in this oral gavage study. There were no significant differences in maternal body weights that could confound exposure. An appropriate negative control condition was utilized (the corn oil vehicle). The negative control response was generally acceptable. Despite the purpose of the paper investigating an endocrine disrupting chemical, the authors do not detail any methods to minimize outside exposure to other plasticizers. Exposure to other plasticizers could impact negative control response and confound the study. There was no positive control condition in this study.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Results were reported for all prespecified outcomes, exposure groups and evaluation time points.

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Study Citation:	Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., Nellemann, C., Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reproductive Toxicology 31(2):200-209.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Dam body weights and weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (14/15 days)-F0- lactation (17)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	806135

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	Test chemical identity, test substance source, and test substance purity were all reported. The purity reported appears to be provided by the vender. The preparation and storage of the chemical was not adequately reported; the authors do state that they use corn oil as a vehicle but no additional information on preparation is given. Gavage volume was not provided. Nominal dose concentration was reported but the authors do not use any analytical methods to confirm the doses. The minor uncertainties about precision of dose levels do not impact interpretation of results.
	Metric 7:	Exposure timing, frequency, and duration	Low	The authors did not attempt to justify the exposure frequency and timing that they used. It is unclear if the exposure timing, duration, and frequency was appropriate for this study, as the authors do not provide justification and all the results for the nutritional endpoints are negative.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The number of exposure groups and dose range/spacing is appropriate for the purposes of this study and based on previously published literature. The outcome assessment methodology (maternal weight, maternal weight gain) was appropriate given the author's interest in the reproductive effects of DINP. The outcome assessments were consistently applied across experimental groups.
	Metric 9:	Results presentation	High	Statistical methods used were clearly described and appropriate for the study. Data for each outcome was reported as mean +/- standard deviation.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Clewell, R. A., Thomas, A., Willson, G., Creasy, D. M., Andersen, M. E. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reproductive Toxicology 35(Elsevier):70-80.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Reproductive/Developmental-Number of litters; number of live pups; number of live pups/litter; male pup body weight; anogenital distance (absolute and scaled = AGD/BW^1/3); average number of nipples/areolae per male pup (based on visual identification; no histology confirmation); testis testosterone level in pups; gubernacular cord length in pups; gross necropsy of pups; examination of genital tract of pups for alterations (e.g., hypospadias, cleft phallus); examination of urogenital tract of pups; examinations for undescended testes and epididymal agenesis in pups; in situ examination of testes, epididymides, gubernacular cords, vas deferens, seminal vesicles, levator ani plus bulbocavernosus (LABC) muscles, and prostate in pups; examination of non-reproductive tissues in situ in pups; organ weights (absolute and relative to body weight) in pups for the following: testis (right and left), epididymis (right and left), seminal vesicle, ventral prostate, glans penis, LABC muscles, Cowper's glands, adrenal gland, kidney (pair), liver; histopathology of testes and epididymides in pups; metabolites of DINP in plasma collected from PND2 pups
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-23)-F0- lactation (PND 0-14)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325348

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported. The test animal species, test article identity, dose levels tested and duration of exposure, route, quantitative or qualitative results for all endpoints examined, parity, commercial animal source, strain, age, sex, starting body weight, animal husbandry conditions (temperature, light/dark cycle, and humidity), number of animals per cage, availability of food and water, test substance source, purity, method of administration, frequency of exposure, number of animals per group, and assays and procedures used to measure endpoints were reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The allocation method for the assignment of pregnant females to experimental groups is not described. There is no other mention of steps that may have been taken to balance variables, such as test animal characteristics or other modifying factors, across experimental groups when assigning animals to experimental groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study implemented methods to reduce observational bias. The study report indicates that the study methodology included "blinding of all observations to ensure objectivity and eliminate bias." The use of blinded observers (which were provided no information on treatment group) was described for specific steps of the endpoint assessment methodology, including for the determination of nipple retention and measurement of anogenital distance. Histopathological examinations of testes and epididymides were conducted using a semi-blinded method of evaluation. For this approach, the initial histopathological examinations of tissues collected from all animals were performed by a primary pathologist with knowledge of positive and negative control groups so that potential changes related to test chemical administration could be identified. The initial examinations were followed by a secondary histopathological examination of all collected tissues by a peer reviewing pathologist who was blinded to the treatment groups (no knowledge of the treatment groups). The approaches used for blinding for histopathological examinations appear to be appropriate for this study.

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Study Citation:	Clewell, R. A., Thomas, A., Willson, G., Creasy, D. M., Andersen, M. E. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reproductive Toxicology 35(Elsevier):70-80.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Reproductive/Developmental-Number of litters; number of live pups; number of live pups/litter; male pup body weight; anogenital distance (absolute and scaled = AGD/BW^1/3); average number of nipples/areolae per male pup (based on visual identification; no histology confirmation); testis testosterone level in pups; gubernacular cord length in pups; gross necropsy of pups; examination of genital tract of pups for alterations (e.g., hypospadias, cleft phallus); examination of urogenital tract of pups; examinations for undescended testes and epididymal agenesis in pups; in situ examination of testes, epididymides, gubernacular cords, vas deferens, seminal vesicles, levator ani plus bulbocavernosus (LABC) muscles, and prostate in pups; examination of non-reproductive tissues in situ in pups; organ weights (absolute and relative to body weight) in pups for the following: testis (right and left), epididymis (right and left), seminal vesicle, ventral prostate, glans penis, LABC muscles, Cowper's glands, adrenal gland, kidney (pair), liver; histopathology of testes and epididymides in pups; metabolites of DINP in plasma collected from PND2 pups
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-23)-F0- lactation (PND 0-14)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325348

Domain	Metric	Rating	Comments
Domain 3: Confounding / Variable Control			
	Metric 4: Confounding / Variable Control	Low	Differences were observed across the study groups that could bias the results and introduce a variable not accounted for in the study analysis. Based on food consumption data, compared to the control group, food consumption was significantly reduced in maternal animals in the high-dose DINP group during gestation (based on mean for GD 13-20; decreased by 17%) and the postnatal period (based on mean for PND 2-14; decreased by 28%). Food consumption was also significantly reduced in the mid-dose group during the postnatal period (based on mean for PND 2-14; decreased by 11%). The study authors attributed the decreases in maternal body weight and body weight gain during gestation and lactation to test substance palatability issues. No other potential confounding variables were identified for maternal animals. The animal husbandry conditions and test substance administration conditions were otherwise consistent across the study 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. Similarly, it is unclear if glass or plastic water bottles were used. Again, plastic bottles could leach phthalates, potentially confounding results. The negative control response was adequate for the endpoints assessed. A positive control group (DBP) was included, which also responded appropriately.
Domain 4: Selective Reporting and Attrition			

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Study Citation:	Clewell, R. A., Thomas, A., Willson, G., Creasy, D. M., Andersen, M. E. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reproductive Toxicology 35(Elsevier):70-80.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Reproductive/Developmental-Number of litters; number of live pups; number of live pups/litter; male pup body weight; anogenital distance (absolute and scaled = AGD/BW^1/3); average number of nipples/areolae per male pup (based on visual identification; no histology confirmation); testis testosterone level in pups; gubernacular cord length in pups; gross necropsy of pups; examination of genital tract of pups for alterations (e.g., hypospadias, cleft phallus); examination of urogenital tract of pups; examinations for undescended testes and epididymal agenesis in pups; in situ examination of testes, epididymides, gubernacular cords, vas deferens, seminal vesicles, levator ani plus bulbocavernosus (LABC) muscles, and prostate in pups; examination of non-reproductive tissues in situ in pups; organ weights (absolute and relative to body weight) in pups for the following: testis (right and left), epididymis (right and left), seminal vesicle, ventral prostate, glans penis, LABC muscles, Cowper's glands, adrenal gland, kidney (pair), liver; histopathology of testes and epididymides in pups; metabolites of DINP in plasma collected from PND2 pups
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-23)-F0- lactation (PND 0-14)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325348

Domain	Metric	Rating	Comments
	Metric 5: Selective Reporting and Attrition	Medium	The study reports the results for all prespecified outcomes, exposure groups, and evaluation timepoints described in the test methods. The number of animals per exposure group is not explicitly stated in the study methods; a range of 20-24 litters/treatment group is indicated in the methods. The results (e.g., Tables 1, 2, and 4, table footnotes) imply that 24 and 20 dams were treated in the control group and test substance-exposed groups, respectively. There are inconsistencies in the numbers of male pups examined on PND 2 across the control and DINP treatment groups without an explanation. The methods describe the selection of 1 male animal per litter on PND 2, but results for fewer than 24 control and 20 test substance-exposed groups are reported for some endpoints. For example, in Table 2, the number of animals was n = 19 for control animals and n = 16 for DINP treatment groups for pup testis and epididymis weights measured on PND 2, whereas 24 control litters and 20 DINP treatment litters are reported for other endpoints. Table 2 also indicates below the table that there were n = 25 control litters "unless otherwise noted", but the number of control litters is reported as 24 in Row 1 of the table. Aside from these inconsistencies, no additional deficiencies were identified. There were no health outcomes identified (e.g., infection) that were unrelated to the exposure that would influence the outcome assessment. No other discrepancies or unexplained omissions or attrition were identified that are expected to affect the interpretation of the results of the study.

Domain 5: Exposure Methods Sensitivity

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Reproductive/Developmental-Number of litters; number of live pups; number of live pups/litter; male pup body weight; anogenital distance (absolute and scaled = AGD/BW^1/3); average number of nipples/areolae per male pup (based on visual identification; no histology confirmation); testis testosterone level in pups; gubernacular cord length in pups; gross necropsy of pups; examination of genital tract of pups for alterations (e.g., hypospadias, cleft phallus); examination of urogenital tract of pups; examinations for undescended testes and epididymal agenesis in pups; in situ examination of testes, epididymides, gubernacular cords, vas deferens, seminal vesicles, levator ani plus bulbocavernosus (LABC) muscles, and prostate in pups; examination of non-reproductive tissues in situ in pups; organ weights (absolute and relative to body weight) in pups for the following: testis (right and left), epididymis (right and left), seminal vesicle, ventral prostate, glans penis, LABC muscles, Cowper's glands, adrenal gland, kidney (pair), liver; histopathology of testes and epididymides in pups; metabolites of DINP in plasma collected from PND2 pups
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-23)-F0- lactation (PND 0-14)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325348

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The study report adequately characterizes the exposure and the administration methods for control and test substance-exposed groups. There are minor uncertainties in the test substance characterization and test diet preparation methods that are expected to have minimal impact on interpretation of the results. The test substance source, purity, and composition were adequately characterized. The purity was reported by the manufacturer and a trade name was provided; however, there is no indication that purity was independently verified. Composition ranges of the diester phthalates (based on alkyl side chain lengths) in the test substance were reported but the manufacturer lot number was not provided. Test diets containing DINP were prepared by adding neat DINP to rodent feed. The study report does not indicate how frequently test diets were prepared, certain conditions of methods used for mixing test substance into feed (e.g., mixing temperature), or conditions of storage of prepared test diets. However, homogeneity of test substance in prepared test diets, test substance stability in feed, and achieved concentrations of test substance in feed were analytically confirmed in samples collected from prepared feed batches. Measured concentrations of test substance in feed were within 3% of target concentrations. Test substance concentrations in samples collected from prepared feed batches at the conclusion of the study (3 months post-mixing) were within 8% of initial concentrations. Average maternal doses of test substance (in mg/kg/day) were calculated from maternal body weight and feed consumption data.

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Study Citation:	Clewell, R. A., Thomas, A., Willson, G., Creasy, D. M., Andersen, M. E. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reproductive Toxicology 35(Elsevier):70-80.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Reproductive/Developmental-Number of litters; number of live pups; number of live pups/litter; male pup body weight; anogenital distance (absolute and scaled = AGD/BW^1/3); average number of nipples/areolae per male pup (based on visual identification; no histology confirmation); testis testosterone level in pups; gubernacular cord length in pups; gross necropsy of pups; examination of genital tract of pups for alterations (e.g., hypospadias, cleft phallus); examination of urogenital tract of pups; examinations for undescended testes and epididymal agenesis in pups; in situ examination of testes, epididymides, gubernacular cords, vas deferens, seminal vesicles, levator ani plus bulbocavernosus (LABC) muscles, and prostate in pups; examination of non-reproductive tissues in situ in pups; organ weights (absolute and relative to body weight) in pups for the following: testis (right and left), epididymis (right and left), seminal vesicle, ventral prostate, glans penis, LABC muscles, Cowper's glands, adrenal gland, kidney (pair), liver; histopathology of testes and epididymides in pups; metabolites of DINP in plasma collected from PND2 pups
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-23)-F0- lactation (PND 0-14)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325348

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure was sensitive for the outcomes of interest for this study. Methods used for exposure administration were consistent across the treatment groups. The period of exposure for this developmental toxicity study did not include exposure timing appropriate for assessing effects on implantation or organogenesis (i.e., dosing period for this study: GD 12 to PND 14; implantation in rats: GD 5; period of organogenesis in rats: GD 5-15). However, the study was designed to examine several endpoints of male rat sexual development in offspring that had been exposed during late gestation and during lactation, recognizing that other referenced studies were available which evaluated test substance-related developmental effects in rodents following exposure during a larger window of the gestational period. The exposure duration was considered appropriate for the intended purpose of this study and the failure to expose the animals during the full period of organogenesis or prior to implantation is not considered a study deficiency.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The procedures were sensitive and specific for evaluating the endpoints and outcomes of interest. The number of exposure groups and dose concentration spacing were appropriate. The test animals were from a commercial source and the species, strain, and sex were appropriate for the evaluation of the intended outcomes. The number of animals per study group was appropriate for the outcome analysis and consistent with studies of similar type. The selection of dose levels was not explicitly justified but the doses tested and the dose level spacing appear appropriate based on the available test data on the developmental effects for this test substance cited by the study authors. The outcome assessment methodology was appropriate to address the outcomes of interest and the outcome assessment was consistent across study groups. The study authors noted that due to the large numbers of animals in the study, all animals could not be necropsied on the same day. Therefore, animals were divided into five necropsy groups, each containing four to five litters from the control group and four litters from each of the test substance exposure groups. The necropsies were divided over two days and the five necropsy groups were treated identically (including the same acclimatization, dosing, and housing conditions). All five of the treatment groups were represented on each necropsy day. The approaches described for necropsy methods appear to be appropriate.

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Study Citation:	Clewell, R. A., Thomas, A., Willson, G., Creasy, D. M., Andersen, M. E. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reproductive Toxicology 35(Elsevier):70-80.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight, body weight gain, food consumption-Reproductive/Developmental-Number of litters; number of live pups; number of live pups/litter; male pup body weight; anogenital distance (absolute and scaled = AGD/BW^1/3); average number of nipples/areolae per male pup (based on visual identification; no histology confirmation); testis testosterone level in pups; gubernacular cord length in pups; gross necropsy of pups; examination of genital tract of pups for alterations (e.g., hypospadias, cleft phallus); examination of urogenital tract of pups; examinations for undescended testes and epididymal agenesis in pups; in situ examination of testes, epididymides, gubernacular cords, vas deferens, seminal vesicles, levator ani plus bulbocavernosus (LABC) muscles, and prostate in pups; examination of non-reproductive tissues in situ in pups; organ weights (absolute and relative to body weight) in pups for the following: testis (right and left), epididymis (right and left), seminal vesicle, ventral prostate, glans penis, LABC muscles, Cowper's glands, adrenal gland, kidney (pair), liver; histopathology of testes and epididymides in pups; metabolites of DINP in plasma collected from PND2 pups
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-23)-F0- lactation (PND 0-14)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	1325348

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	The results presentation was appropriate for the outcomes of interest and endpoints evaluated. The statistical analyses methods were clearly described and appropriate for the data sets evaluated. Quantitative data for the reported effects were reported with means and SE or SD values for continuous data and incidences were provided for categorical data (e.g., histopathology of pups) including reporting of the numbers of animals affected and the total numbers of animals examined.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Furr, J. R., Lambright, C. S., Wilson, V. S., Foster, P. M., Gray, L. E., Jr (2014). A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. Toxicological Sciences 140(2):403-424.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD14- GD18)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2510906 Linked HERO ID(s): 2510906, 3045543

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	Good. Important information is provided for test animals, exposure methods, experimental design, endpoint evaluations, and the presentation of results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Adequate. Pregnant rats were randomly assigned to treatment groups on GD 14 in a manner that provided each group with similar means and variances in body weight. The method for randomization is not detailed, but this description indicates that normalization 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 Control				
	Metric 4:	Confounding / Variable Control	High	Good. Vehicle (laboratory grade corn oil) and gavage volume were the same in control and treatment groups. Additionally, water was tested monthly for Pseudomonas and every four months for a suite of chemicals, including pesticides and heavy metals. The experimental conditions described provided no indication of different practices across treatment groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Adequate. All endpoints described in methods were reported qualitatively or quantitatively. Data are complete for all endpoints (generally 3-4 dams per group).
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Adequate. The authors tested several "blocks" of animals, and the source, purity, and lot # was reported for each block. Chemicals were supplied by Aldrich and BASF were $\geq$99% pure in all cases, although it is not clear that the authors independently verified the chemical purity or stability. Dams were weighed and dosed daily with test chemical in laboratory grade corn oil.

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Study Citation:	Furr, J. R., Lambright, C. S., Wilson, V. S., Foster, P. M., Gray, L. E., Jr (2014). A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. Toxicological Sciences 140(2):403-424.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD14- GD18)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2510906 Linked HERO ID(s): 2510906, 3045543

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	Testosterone: Good. Pregnant dams were dosed daily with test substance 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Testosterone: Good. 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. One testis each was dissected from 3 male fetuses/litter; it is not clear whether the individual testes 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 testosterone production, although authors stated that changes less than 20-25% may not be consistently detected.
	Metric 9:	Results presentation	High	All outcomes: Good. There are no notable concerns about the way the results are analyzed or presented.

Additional Comments: Testosterone: High confidence. This study was well-designed to evaluate effects on fetal testicular testosterone. The sample size was small, but was validated by authors to have sufficient statistical power for this analysis. Evidence was presented clearly and transparently.

Overall Quality Determination High

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 Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl 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.
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 Control				
	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, plastic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophosphates. No analysis of food for potential endocrine disruptors was conducted.

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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 Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments
Domain 4: Selective Reporting and Attrition			
	Metric 5: Selective Reporting and Attrition	Medium	The text states that there was no mortality, overt toxicity, or reduced maternal body weight or reduced litter size at any of the tested doses, indicating no attrition for other endpoints The methods state that there were 3-6 dams group treated with DINP CASRN 28553-12-0 and 3/group treated with CASRN 68515-48-0. There were no dam mortalities. Data from both CASRNs were combined in Table 1; the sample sizes for two dose groups (500 and 750 mg/kg-day), were n=5 litters. Based on the dams treated/group, a minimum of 6 litters was expected. It is unclear whether there was any unreported animal attrition, or if this represents an outlier, or selective reporting. No author justification was provided.
Domain 5: Exposure Methods Sensitivity			
	Metric 6: Chemical administration and characterization	Low	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported and could not be determined. The test substance was a gift from Badische Anilin and Soda Fabeik and was not independently analyzed by the performing laboratory The study did not measure concentration in corn oil or report if doses were prepared fresh. No details of preparation (e.g., 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.
	Metric 7: Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. Pregnant 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 Measures and Results Display			

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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 Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	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, which may have reduced the accuracy of the results. 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 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. Overall, the sample size was small (n=3 dams/dose group), which may reduce the sensitivity or statistical power.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 testosterone production, although authors stated that changes less than 20-25% may not be consistently detected (see Furr et al. 2014 [2510906]).
	Metric 9:	Results presentation	Low	Data for DINP (CAS RN 28553-12-0) and DINP (mixed isomers; CAS RN 68515-48-0) were not significantly different, therefore study authors combined the data from the two chemicals in Table 1 (mean testosterone production (with SE). The study does report data on the two chemicals independently in Figure 6. This figure shows testosterone production as percentage of control, and supports the authors claim that responses from the two chemicals were similar. Figure 6 does not report the number of animals for each chemical; Table 1 reports a combined number of animals.

Additional Comments: None

Overall Quality Determination 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 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):	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl 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.
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 Control				
	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, plastic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophosphates. No analysis of food for potential endocrine disruptors was conducted.
Domain 4: Selective Reporting and Attrition				

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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 Health Effect(s):	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The text states that there was no mortality, overt toxicity, reduced maternal body weight or reduced litter size at any of the tested doses, indicating no attrition for other end-points. The number of pregnant dams reported in the methods is in agreement with the numbers assessed (as reported in Table 1). The methods state that there were 3-6 dams group treated with DINP CASRN 28553-12-0 and 3/group treated with CASRN 68515-48-0. There were no dam mortalities. Data from both CASRNs were combined in Table 1; the sample sizes for two dose groups (500 and 750 mg/kg-day), were n=5 litters. Based on the dams treated/group, a minimum of 6 litters was expected. It is unclear whether there was any unreported animal attrition, or if this represents an outlier, or selective reporting. No author justification was provided.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported and could not be determined. The test substance was a gift from Badische Anilin and Soda Fabeik and was not independently analyzed by the performing laboratory. The study did not measure the concentration in corn oil or report if doses were prepared fresh. No details of preparation (e.g., homogeneity) or stability were provided. It is not reported whether doses were adjusted daily based on maternal body weight.
	Metric 7:	Exposure timing, frequency, and duration	Medium	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 Measures and Results 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 publication 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 adequate to consistently detect anything other than rather large alterations of maternal weight gain. Clinical signs: The authors did not report how often animals were assessed for clinical signs of toxicity.
	Metric 9:	Results presentation	Medium	Data were reported as negative in the text.

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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 Health Effect(s):	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination 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 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)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl 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.
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 Control				
	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, plastic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophosphates. No analysis of food for potential endocrine disruptors was conducted.
Domain 4: Selective Reporting and Attrition				

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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 Health Effect(s):	Mortality-Mortality (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	The text states that there was no mortality, overt toxicity, or reduced maternal body weight or reduced litter size at any of the tested doses, indicating no attrition for other endpoints. The number of pregnant dams reported in the methods is in agreement with the numbers assessed (as reported in Table 1).
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The route and gavage volume were appropriate. The purity or grade of the test substance was not reported and could not be determined. The test substance was a gift from Badische Anilin and Soda Fabeik and was not independently analyzed by the performing laboratory. The study did not measure the concentration in corn oil or report if doses were prepared fresh. No details of preparation (e.g., homogeneity) or stability were provided. It is not reported whether doses were adjusted daily based on maternal body weight.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. Pregnant 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The study did not report how often animals were assessed for mortality; however, no deaths occurred. The lack of this information does not affect the ability to assess this endpoint. The animal model was appropriate for the outcome of interest. The sample size was small (n= 3 dams/group), but this is not expected to have a significant impact on this outcome.
	Metric 9:	Results presentation	Medium	Data were reported as negative in the text.
Additional Comments:	None			

Overall Quality Determination — Medium

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):	Mortality-Maternal lethality
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl 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 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 Attrition				
	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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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):	Mortality-Maternal lethality
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	674193 Linked 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 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 beginning 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 Measures and Results 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 animals/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 Quality Determination Medium

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):	Reproductive/Developmental-Reproductive: Uterus weight, corpora lutia/dam, implantations sites/dam, placental weight; Developmental: pre and post implantation loss, total resorptions, live fetuses, fetal weights, fetal and skeletal variations and malformations
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl 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 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 inconsistent. No other confounding variables were reported.
Domain 4: Selective Reporting and Attrition				
	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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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):	Reproductive/Developmental-Reproductive: Uterus weight, corpora lutia/dam, implantations sites/dam, placental weight; Developmental: pre and post implantation loss, total resorptions, live fetuses, fetal weights, fetal and skeletal variations and malformations
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	674193 Linked 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 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 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 beginning 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 Measures and Results 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 variance. 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 Quality Determination Medium

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):	Other (please specify below) (Clinical signs)-Maternal clinical signs-Nutritional/Metabolic-Maternal body weights, food consumption, body weight change
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl 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 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 Attrition				
	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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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):	Other (please specify below) (Clinical signs)-Maternal clinical signs-Nutritional/Metabolic-Maternal body weights, food consumption, body weight change
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	674193 Linked 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 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 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 beginning 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, duration, and frequency were adequate for the selected outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	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 those specified in OECD TG 414.
	Metric 9:	Results presentation	Low	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 were appropriate.

Additional Comments: None

Overall Quality Determination Medium

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:	Diisononyl 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 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 Attrition				
	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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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:	Diisononyl Phthalate- Parent compound
HERO ID:	674193 Linked 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-dimethylheptanol-1. The nominal doses were calculated based on animal body weights at the beginning 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, duration, and frequency were adequate for the selected outcomes of interest.
Domain 6: Outcome Measures and Results 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

Overall Quality Determination Medium

Study Citation:	Jr, Gray, L. E., Lambright, C. S., Evans, N., Ford, J., Conley, J. M. (2024). Using targeted fetal rat testis genomic and endocrine alterations to predict the effects of a phthalate mixture on the male reproductive tract. Current Research in Toxicology 7:100180.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ex vivo fetal testicular testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-18)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11785000

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered High for Domain 1. All critical and most important information was reported. The test animals' species, chemical name, doses, duration of exposure, and route of exposure were clearly reported, and quantitative results were provided for at least one endpoint. The test animal source, strain, age, sex, and starting body weight were reported. Parity status was not reported. Information on animal husbandry was reported, including temperature, humidity, light/dark cycle, diet, water availability, and number of animals per cage. The test substance source and purity and the method of administration were reported. The frequency of exposure, number of animals per study group, animal age and life stage during exposure and at endpoint/outcome evaluation were also reported. Assays used to evaluate the outcome of interest were also reported. The only missing piece of important information was the parity status of the animals; however, this is not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for Domain 2.1. The dams were weight-ranked and randomly assigned to treatment groups using experimental design software.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered Medium for Domain 2.2. Measures to reduce observational bias were not described, but the potential concern for bias was mitigated because the outcomes were not subjective and based on the use of automated/computer-driven systems (LC-MS).
Domain 3: Confounding / Variable Control				

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Study Citation:	Jr, Gray, L. E., Lambright, C. S., Evans, N., Ford, J., Conley, J. M. (2024). Using targeted fetal rat testis genomic and endocrine alterations to predict the effects of a phthalate mixture on the male reproductive tract. Current Research in Toxicology 7:100180.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ex vivo fetal testicular testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-18)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11785000

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	Low	This study is considered Low for Domain 3. The negative control group was exposed to corn oil vehicle only in the same manner as the treated groups (via gavage). A positive control group was not included and is not required. The animals were randomized based on body weight at the beginning of the study, so there is no concern for differences in initial body weight. Food/water intake was not reported; however, there were no statistically significant differences in body weight or weight gain between the treated and control groups. Palatability is also not an issue, as the test substance was administered via gavage. Animal husbandry conditions were well-described and uniform across all groups; however, the animals were housed in polycarbonate cages. 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. Food and water dispensing containers were not described. The potential for co-exposure to plasticizers is a major confounding factor.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	This study is considered High for Domain 4. The number of animals in the control and treatment groups were identified as n=7-8 per group. Although survival was not explicitly stated, the individual data were available in the supplemental materials. Additionally, although it was not explicitly stated in the results that 3 testis per litter per treatment group were collected (as per the methods section), the individual data were available in the supplemental materials, confirming there was no attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	This study is considered Medium for Domain 5.1. The test substance and vehicle were identified and the source and lot of each were provided. The purity of the test substances was reported (DBP= 99.9% and DINP=99%) There was no indication that the test substance was verified by the performing laboratory. Gavage volume was reported to be 2.5 ml/kg-body weight. Dosing occurred at the same time each day. Doses were reported in mg/kg-day. It was not specified whether the doses were adjusted daily based on dam body weight. The test substance was prepared in corn oil, but no other details were provided (how solutions were mixed, frequency solutions were made). No details on the storage or stability of the test solutions were provided. Although some details in reporting are lacking, there is no indication that these omissions are likely to have a substantial impact on the study evaluation.

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Study Citation:	Jr, Gray, L. E., Lambright, C. S., Evans, N., Ford, J., Conley, J. M. (2024). Using targeted fetal rat testis genomic and endocrine alterations to predict the effects of a phthalate mixture on the male reproductive tract. Current Research in Toxicology 7:100180.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ex vivo fetal testicular testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-18)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	11785000

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	This study is considered Medium for Domain 5.2. The purpose of this study was to measure fetal testicular testosterone; however, animals were dosed from GD 14-GD 18 and sacrificed on GD 18. Fetal testicular testosterone is produced between GD 14-GD 21, so the early sacrifice may not have captured the true fetal testicular testosterone level. However, as the exposure covered most of the critical window, and the control animals were also sacrificed at GD 18, the early sacrifice is considered a minor limitation. The route and frequency (daily gavage exposure between 0700 and 0900 EST) were appropriate for the study type and outcome of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The study is considered Medium for Domain 6.1. The doses were justified by the authors, but only one dose per chemical was used. Outcome assessment methodologies were sensitive for the outcomes of interest and were consistently assessed across groups. The test animals selected were appropriate. The sample size (n=7 pregnant females/group) is slightly lower for the DBP and DINP groups than what is recommended by OECD for a reproductive study (n=8 pregnant females/group).
	Metric 9:	Results presentation	High	The study is considered High for Domain 6.2 for the reproductive/developmental endpoint. Data were analyzed and presented appropriately and included statistical significance. Individual animal data were provided in the supplemental file. Each litter was considered the experimental unit.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Birth rate, % male pups, male pup body weights, length, AGD, pup testes testosterone levels, testes immunohistochemistry, histopathology, measurements of testes volume and Legdig cell number and size, gene expression analysis.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study included all critical information and all important information. The test substance was identified as DINP; the source purity and composition were reported. Provided information included the test animals (Sprague Dawley) sex, and source. The dam body weights prior to exposure were reported. Parity was not specified, but this study was not assessing reproductive function. Reported animal husbandry details included temperature, humidity, light cycle, number of animals per cage and details on bedding, food type, and water and food availability. Details of test substance administration, number of animals per group, doses, endpoint evaluation methods, and quantitative results for at least one endpoint were provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly distributed into study groups; however, the method of randomization was not specified. It is unclear if animals were normalized to body weights. It was noted that three sets of fetal testes (at least one per dam) were randomly selected for downstream analysis, and computer-assisted image analysis of testes tissues was conducted on 8 randomly selected fields.
	Metric 3:	Observational Bias / Blinding Changes	Medium	The study did not specify blinding; however, the concern for bias was mitigated for most of the outcomes because they were not subjective and/or were based on the use of automated/computer-driven systems, standard laboratory kits, simple objective measures (e.g., body or tissue weight), or screening-level evaluations of histopathology or quantitative immunohistochemistry.
Domain 3: Confounding / Variable Control				

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Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Birth rate, % male pups, male pup body weights, length, AGD, pup testes testosterone levels, testes immunohistochemistry, histopathology, measurements of testes volume and Legdig cell number and size, gene expression analysis.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	Medium	The study included a concurrent negative vehicle (corn oil) control. The control responses were appropriate. A positive control was not necessary for the study type. Food and water consumption were not reported in a gavage study, but the impact on results is expected to be minimal. Dam body weights were not reported. Animal husbandry details were provided and conditions were consistent across groups. The study did not address the possibility of co-exposures to plasticizers. Animals were held individually in IVC cages, but the cage material, and materials used for gavage or to hold water were not specified and food was not analyzed for contaminates. Although some details were missing, based on the information provided, there was no evidence of confounding variables. The fetuses from one dam that died during pregnancy were surgically removed and were alive. It is unclear whether these were included in the datasets for male pups, or if the method of delivery had any impact on the study results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Qualitative or quantitative results were reported for all specified outcomes. The study reported the number of animals per group, and the sample sizes were noted in the text or table/figure legends. One dam at the high dose died during pregnancy. The cause of death was not specified, and the viable fetuses were extracted from this dam.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test substance was DINP ($\geq$99% mixture of C9 isomers with $\leq$ 0.15% dioctyl phthalate) purchased from Sigma. The test substance was not analytically verified by the testing laboratory, but Sigma only sells a single DINP product and a certificate of analysis can be obtained. The reported doses are presumed to be nominal. There is no indication that concentrations in the dosing solutions were measured analytically. The test substance was dissolved in corn oil; no further details on the preparation of the test solutions (including assurance of homogeneity, frequency of preparation, or stability), or storage were provided. The gavage volume was not reported, but gavage is an appropriate route of exposure for this test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed daily from GD 12 to GD 21. The exposure window was justified by the study authors; the time was consistent with Leydig cell development and was appropriate for the purposes of the study.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Birth rate, % male pups, male pup body weights, length, AGD, pup testes testosterone levels, testes immunohistochemistry, histopathology, measurements of testes volume and Legdig cell number and size, gene expression analysis.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	The dose spacing was not explicitly justified by the study authors, and a NOAEL was not determined. However, the doses were within the range of several cited studies that also looked at the reproductive effects of DINP exposure. Outcome assessment methodologies were sensitive to the outcomes of interest. Sufficient details on the outcome assessment protocols were provided. Some details are available in supplementary files. Justification for the test species/strain was not provided; however, Sprague-Dawley rats were an appropriate model selection. The study used 6 animals per group which allowed for statistical analysis. The number of testis sections examined for each endpoint was described.
	Metric 9:	Results presentation	Medium	The methods specified that pup lengths were measured, but these results were not reported. All other data were adequately reported as means ± SEM, where relevant, in tables and figures. The sample sizes and statistical significance were noted. The methods of statistical analysis were reported and were appropriate for most datasets. It was not specified whether the litter endpoints (body weights, % males, AGDs) were analyzed using the litter as the unit of statistical analysis; the methods specified that the number of male pups was used. Individual animal data were not provided.

Additional Comments: The supplemental files cited in the paper are not available on PubMed. The study authors are being contacted. The current evaluation was conducted without viewing the supplemental files.

Overall Quality Determination Medium

Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Dam mortality
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study included all critical information and all important information. The test substance was identified as DINP; the source purity and composition were reported. Provided information included the test animals (Sprague Dawley) sex, and source. The dam body weights prior to exposure were reported. Parity was not specified, but this study was not assessing reproductive function. Reported animal husbandry details included temperature, humidity, light cycle, number of animals per cage and details on bedding, food type, and water and food availability. Details of test substance administration, number of animals per group, doses, endpoint evaluation methods, and quantitative results for at least one endpoint were provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly distributed into study groups; however, the method of randomization was not specified. It is unclear if animals were normalized to body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	The study did not specify blinding; however, the concern for bias was mitigated because mortality and body weights are not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a concurrent negative vehicle (corn oil) control. The control responses were appropriate. A positive control was not necessary for the study type. Food and water consumption were not reported in a gavage study, but the impact on results is expected to be minimal. Dam body weights were not reported. Animal husbandry details were provided and conditions were consistent across groups. The study did not address the possibility of co-exposures to plasticizers. Animals were held individually in IVC cages, but the cage material, and materials used for gavage or to hold water were not specified and food was not analyzed for contaminates. Although some details were missing, based on the information provided, there was no evidence of confounding variables.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Qualitative or quantitative results were reported for all specified outcomes. The study reported the number of animals per group, and the sample sizes were noted in the text or table/figure legends. One dam at the high dose died during pregnancy. The cause of death was not specified, so it cannot be determined if this death was due to attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Dam mortality
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test substance was DINP (≥99% mixture of C9 isomers with ≤ 0.15% dioctyl phthalate) purchased from Sigma. The test substance was not analytically verified by the testing laboratory, but Sigma only sells a single DINP product and a certificate of analysis can be obtained. The reported doses are presumed to be nominal. There is no indication that concentrations in the dosing solutions were measured analytically. The test substance was dissolved in corn oil; no further details on the preparation of the test solutions (including assurance of homogeneity, frequency of preparation, or stability), or storage were provided. The gavage volume was not reported, but gavage is an appropriate route of exposure for this test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed daily from GD 12 to GD 21. The exposure window was justified by the study authors; the time was consistent with Leydig cell development and was appropriate for the purposes of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The dose spacing was not explicitly justified by the study authors, and a NOAEL was not determined. However, the doses were within the range of several cited studies that also looked at the reproductive effects of DINP exposure. The methods did not explicitly indicate that dam mortality was being assessed, but one death was reported. Justification for the test species/strain was not provided; however, Sprague-Dawley rats were an appropriate model selection. The study used 6 animals per group which is sufficient to allow for statistical analysis if needed.
	Metric 9:	Results presentation	Medium	A single death was reported in the study text, but the cause of death was not specified.

Additional Comments: The supplemental files cited in the paper are not available on PubMed. The study authors are being contacted. The current evaluation was conducted without viewing the supplemental files.

Overall Quality Determination Medium

Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Dam body weights (prior to dosing) on GD 0 and on GD 11. Dam weight gain after birth.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study included all critical information and all important information. The test substance was identified as DINP; the source purity and composition were reported. Provided information included the test animals (Sprague Dawley) sex, and source. The dam body weights prior to exposure were reported. Parity was not specified, but this study was not assessing reproductive function. Reported animal husbandry details included temperature, humidity, light cycle, number of animals per cage and details on bedding, food type, and water and food availability. Details of test substance administration, number of animals per group, doses, endpoint evaluation methods, and quantitative results for at least one endpoint were provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly distributed into study groups; however, the method of randomization was not specified. It is unclear if animals were normalized to body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	The study did not specify blinding; however, the concern for bias was mitigated because mortality and body weights are not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a concurrent negative vehicle (corn oil) control. The control responses were appropriate. A positive control was not necessary for the study type. Food and water consumption were not reported in a gavage study, but the impact on results is expected to be minimal. Dam body weights were not reported. Animal husbandry details were provided and conditions were consistent across groups. The study did not address the possibility of co-exposures to plasticizers. Animals were held individually in IVC cages, but the cage material, and materials used for gavage or to hold water were not specified and food was not analyzed for contaminates. Although some details were missing, based on the information provided, there was no evidence of confounding variables.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Qualitative or quantitative results were reported for all specified outcomes. The study reported the number of animals per group, and the sample sizes were noted in the text or table/figure legends. One dam at the high dose died during pregnancy. The cause of death was not specified, so it cannot be determined if this death was due to attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Li, L., Bu, T., Su, H., Chen, Z., Liang, Y., Zhang, G., Zhu, D., Shan, Y., Xu, R., Hu, Y., Li, J., Hu, G., Lian, Q., Ge, R. S. (2015). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicology Letters 232(2):466-474.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Dam body weights (prior to dosing) on GD 0 and on GD 11. Dam weight gain after birth.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	2807612

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test substance was DINP (≥99% mixture of C9 isomers with ≤ 0.15% dioctyl phthalate) purchased from Sigma. The test substance was not analytically verified by the testing laboratory, but Sigma only sells a single DINP product and a certificate of analysis can be obtained. The reported doses are presumed to be nominal. There is no indication that concentrations in the dosing solutions were measured analytically. The test substance was dissolved in corn oil; no further details on the preparation of the test solutions (including assurance of homogeneity, frequency of preparation, or stability), or storage were provided. The gavage volume was not reported, but gavage is an appropriate route of exposure for this test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed daily from GD 12 to GD 21. The exposure window was justified by the study authors; the time was consistent with Leydig cell development and was appropriate for the purposes of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The dose spacing was not explicitly justified by the study authors, and a NOAEL was not determined. However, the doses were within the range of several cited studies that also looked at the reproductive effects of DINP exposure. The methods did not explicitly indicate that dam body weights or weight gain were being assessed, but results for these endpoints were provided in a table. Body weights were only reported on GD0 and GD11, prior to the start of exposure, which is not useful for assessing the effects of exposure on this endpoint. Weight gain after birth was reported. Because no methods were provided, it is unclear if this is a weight gain from GD0 til birth, or if it was a gain from GD11 til birth. Justification for the test species/strain was not provided; however, Sprague-Dawley rats were an appropriate model selection. The study used 6 animals per group which is sufficient to allow for statistical analysis if needed.
	Metric 9:	Results presentation	Low	Body weight data and weight gain were reported quantitatively as means ± SEM. Insufficient details of weight gain after birth were provided (it was not specified what time points the reported gain covered). These data are either not informative (body weight measurements only reported prior to the start of exposure) or provide insufficient information to interpret the study results (weight gain after birth).

Additional Comments: The supplemental files cited in the paper are not available on PubMed. The study authors are being contacted. The current evaluation was conducted without viewing the supplemental files.

Overall Quality Determination Medium

Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weight gain and food consumption
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance identity, source, and purity (>98%) were reported. Test animal species, strain, sex, and source were reported. Age, number of pregnancies, 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 randomized into study groups; however, the method used was not reported.
	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, food intake, measurable endpoint) or blinding is not recommended (initial histopathology review).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and appropriate. Animal husbandry conditions were consistent across groups. The food intake of high-dose dams was decreased by >20% throughout most of the study period and may have been due to palatability. This occurred in conjunction with significant reductions in body weight gain and is expected to confound the interpretation of the study results at the high dose. 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. Plastic bottles could leach phthalates that could confound results. Authors did take the precaution of using a soy-fee diet to "eliminate possible estrogenic effects for a standard diet".
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There is no indication of attrition or that treated animals were excluded from analysis.

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Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weight gain and food consumption
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test substance was obtained from WAKO Pure Chemical Industries Ltd with a reported purity of as >98%. It is likely the supplier provided a certificate of analysis; but the test substance was not analytically verified by the performing laboratory. The test substance was administered to the dams through diet. The study does not provide any details on the methods used to add the test substance to the diet, how frequently the diet was prepared, storage conditions of the prepared diet, or verify concentration levels in the diet. This lack of reporting introduces substantial ambiguity about the precision of dose levels. The study authors did measure food consumption and reported estimates of the doses animals received based on food consumption and body weight, but these measurements were not provided in the study report.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing, and duration were appropriate for the study's aim. Dams were exposed from GD 15 to PND 10 which is appropriate since "sexual differentiation of endocrine/reproductive systems, including the neuroendocrine center of the hypothalamus, occurs during the late gestational and neonatal period in rodents (Davies and Norman 2002)."
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The number of exposure groups and spacing was appropriate and justified by the study authors and based on the results of a preliminary study. The outcome methodologies were described with limited details. The frequency of dam body weight and food consumption measurements was not specified. Measurements every three days is recommended for similar study types. Only body weight gain, but not body weights were reported. The number of animals per group and sample sizes for these outcomes were small (n=5), but sufficient for statistical analysis.
	Metric 9:	Results presentation	High	Body weight gain and food consumption were reported with means and SE. Statistical analysis was performed appropriately by study authors.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-On PND2: the number, weights and AGD distance. On PND 27 (5/sex/group): organ weights (brain, adrenals, testes, ovaries, and uterus), brain volume measurement of SDN-POA. Remaining offspring were assessed for age and body weights at onset of puberty, estrous cyclicity (assessed via vaginal smears during PNW8-11).At post-natal week 11: organ weights (brain, adrenals, testes, ovaries, uterus, pituitary, and ventral prostate), histology (pituitary, thyroids, adrenal, mammary gland, epididymites, prostate, seminal vesicle, ovaries, uterus, and vagina), and morphometric analyses of testes (percentage of seminiferous tubules with vacuolation) and ovaries (number of secondary follicles, large atretic follicles, and corpora lutea)
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance identity, source, and purity (>98%) were reported. Test animal species, strain, sex, and source were reported. Age, number of pregnancies, 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 randomized into study groups and pups were culled randomly on PND10. The method(s) of randomization were not reported.
	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, food intake, measurable endpoint) or blinding is not recommended (initial histopathology review).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and appropriate. Animal husbandry conditions were consistent across groups. The food intake of high-dose dams was decreased by >20% throughout most of the study period and may have been due to palatability. This occurred in conjunction with significant reductions in body weight gain and is expected to confound the interpretation of the study results at the high dose. 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. Plastic bottles could leach phthalates that could confound results. Authors did take the precaution of using a soy-fee diet to "eliminate possible estrogenic effects for a standard diet".
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There is no indication of attrition or that treated animals were excluded from analysis.

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Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-On PND2: the number, weights and AGD distance. On PND 27 (5/sex/group): organ weights (brain, adrenals, testes, ovaries, and uterus), brain volume measurement of SDN-POA. Remaining offspring were assessed for age and body weights at onset of puberty, estrous cyclicity (assessed via vaginal smears during PNW8-11).At post-natal week 11: organ weights (brain, adrenals, testes, ovaries, uterus, pituitary, and ventral prostate), histology (pituitary, thyroids, adrenal, mammary gland, epididymites, prostate, seminal vesicle, ovaries, uterus, and vagina), and morphometric analyses of testes (percentage of seminiferous tubules with vacuolation) and ovaries (number of secondary follicles, large atretic follicles, and corpora lutea)
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test substance was obtained from WAKO Pure Chemical Industries Ltd with a reported purity of as >98%. It is likely the supplier provided a certificate of analysis; but the test substance was not analytically verified by the performing laboratory. The test substance was administered to the dams through diet. The study does not provide any details on the methods used to add the test substance to the diet, how frequently the diet was prepared, storage conditions of the prepared diet, or verify concentration levels in the diet. This lack of reporting introduces substantial ambiguity about the precision of dose levels. The study authors did measure food consumption and reported estimates of the doses animals received based on food consumption and body weight, but these measurements were not provided in the study report.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing, and duration were appropriate for the study's aim. Dams were exposed from GD 15 to PND 10 which is appropriate since "sexual differentiation of endocrine/reproductive systems, including the neuroendocrine center of the hypothalamus, occurs during the late gestational and neonatal period in rodents (Davies and Norman 2002)."
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The endpoints evaluated were partially sensitive to outcomes of interest (developmental) and for the purposes of the study. The authors noted that volume measurements of SDN-POA "may not be sensitive enough to detect weak hormonal influence on brain sexual differentiation and raise needs for alternative endpoints, such as behavioral parameters, assessment of gonadotropin-releasing hormone levels, or measurement of new molecular variables, such as region-specific gene expression in the hypothalamus (Shibutani et al., manuscript submitted)." Outcomes were assessed consistently across study groups. The doses chosen were based on previous findings in which the highest dose resulted in moderate toxicity to the dams but did not affect pregnancy, delivery, or lactation. The animal model was appropriate for this study type and obtained from a commercial source. Only a small number of dams were used in the study (n = 5/group), this likely reduced the statistical power for identifying litter effects.

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Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-On PND2: the number, weights and AGD distance. On PND 27 (5/sex/group): organ weights (brain, adrenals, testes, ovaries, and uterus), brain volume measurement of SDN-POA. Remaining offspring were assessed for age and body weights at onset of puberty, estrous cyclicity (assessed via vaginal smears during PNW8-11).At post-natal week 11: organ weights (brain, adrenals, testes, ovaries, uterus, pituitary, and ventral prostate), histology (pituitary, thyroids, adrenal, mammary gland, epididymites, prostate, seminal vesicle, ovaries, uterus, and vagina), and morphometric analyses of testes (percentage of seminiferous tubules with vacuolation) and ovaries (number of secondary follicles, large atretic follicles, and corpora lutea)
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Data were fully reported for most outcomes of interest along with appropriate statistical analysis. The study did not report histological findings for organs other than testis, prostate, epididymis, and ovaries. The study does state in the discussion that no other developmental effects were seen except for the histological changes in the testes, therefore it is reasonable to assume findings were negative for other organs not reported.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Maternal mortality
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance identity, source, and purity (>98%) were reported. Test animal species, strain, sex, and source were reported. Age, number of pregnancies, 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 randomized into study groups and pups were culled randomly on PND10. The method(s) of randomization were not reported.
	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, food intake, measurable endpoint) or blinding is not recommended (initial histopathology review).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and appropriate. Animal husbandry conditions were consistent across groups. The food intake of high-dose dams was decreased by >20% throughout most of the study period and may have been due to palatability. This occurred in conjunction with significant reductions in body weight gain and is expected to confound the interpretation of the study results at the high dose. 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. Plastic bottles could leach phthalates that could confound results. Authors did take the precaution of using a soy-fee diet to "eliminate possible estrogenic effects for a standard diet".
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There is no indication of attrition or that treated animals were excluded from analysis.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192(2-3):149-170.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Maternal mortality
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (GD15-birth)-F0- lactation (birth-PND10)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	192872

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test substance was obtained from WAKO Pure Chemical Industries Ltd with a reported purity of as >98%. It is likely the supplier provided a certificate of analysis; but the test substance was not analytically verified by the performing laboratory. The test substance was administered to the dams through diet. The study does not provide any details on the methods used to add the test substance to the diet, how frequently the diet was prepared, storage conditions of the prepared diet, or verify concentration levels in the diet. This lack of reporting introduces substantial ambiguity about the precision of dose levels. The study authors did measure food consumption and reported estimates of the doses animals received based on food consumption and body weight, but these measurements were not provided in the study report.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing, and duration were appropriate for the study's aim. Dams were exposed from GD 15 to PND 10 which is appropriate since "sexual differentiation of endocrine/reproductive systems, including the neuroendocrine center of the hypothalamus, occurs during the late gestational and neonatal period in rodents (Davies and Norman 2002)."
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The study did not provide any detail on how often animals were checked on; however, no animals died. Lack of this information is unlikely to affect results.
	Metric 9:	Results presentation	Medium	Mortality: The study does not state specifically that no animals died, however it does report "all dams delivered live pups".

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Waterman, S. J., Ambroso, J. L., Keller, L. H., Trimmer, G. W., Nikiforov, A. I., Harris, S. B. (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reproductive Toxicology 13(2):131-136.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Corpora lutea/dam, implantations/dam, resorptions/dam, post-implantation loss %, viable fetuses/dam, fetal body weights, fetal sex distribution, fetuses with malformations, fetuses with variations, total affected fetuses (sum of resorptions, dead and malformed fetuses/litter), % fetuses and % litters with visceral and skeletal variations: including dilated renal pelves, skeletal variations, lumbar ribs, and cervical ribs. Uterus weights.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680201 Linked HERO ID(s): 680201, 679108, 680097, 10750187

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	Test substance name, CASRN, purity and source are reported. Test animal species, strain, sex, parity, source and animal housing conditions (such as number of animals/cage, food and water availability, temperature, humidity and light/dark cycle) were reported. Animal starting age was not reported, and starting animal weight is only reported graphically starting from GD 0 (body weights from the start of the quarantine period after the animals arrived are not reported). Exposure details and endpoint assessment methods were reported in adequate detail.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors stated that animals were assigned to dose groups in the order of mating, which is a non-random method of allocation and the authors do not specify if the allocated animals to one dose group first, or if they assigned mated animals to different groups after the completion of mating. This ambiguity could imply some degree of bias if animals with higher reproductive fitness are disproportionately assigned to one group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No blinding of exposure groups was described, but most endpoints are objective in nature. For data on fetal malformations, no blinding was described, but an external reviewer verified skeletal findings which may minimize potential impacts of observational bias on the results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	An appropriate negative vehicle control was used and there was no response in the control group. The authors measured several factors that may act as confounders (body weights, food consumption) and the gavage route of exposure prevents potential confounding from palatability issues.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The authors state that no dams died. The authors did not state the initial sample size at the start of dosing in the methods but sample size in tables is reported as a range (22-25) in the results, implying that some animals or litters may be excluded from the analysis. The authors do not explain this omission, but it is unlikely to have a large impact on the results. Most endpoints described in the methods appear in the results, with the exception of uterus weights that were described in the methods but do not appear anywhere in the results. The significance of this omission is relatively minor.

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Study Citation:	Waterman, S. J., Ambroso, J. L., Keller, L. H., Trimmer, G. W., Nikiforov, A. I., Harris, S. B. (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reproductive Toxicology 13(2):131-136.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Corpora lutea/dam, implantations/dam, resorptions/dam, post-implantation loss %, viable fetuses/dam, fetal body weights, fetal sex distribution, fetuses with malformations, fetuses with variations, total affected fetuses (sum of resorptions, dead and malformed fetuses/litter), % fetuses and % litters with visceral and skeletal variations: including dilated renal pelves, skeletal variations, lumbar ribs, and cervical ribs. Uterus weights.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680201 Linked HERO ID(s): 680201, 679108, 680097, 10750187

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test substance is identified definitively by name and CASRN and the reported purity is sufficient. The authors performed their own independent analytical verification of the test substance purity using an acceptable method. There are no concerns regarding test substance preparation. The gavage volume is acceptable, and the route of exposure is appropriate for the COI. Test substance storage conditions were not described, but the COI has high stability, and the exposure period was relatively short, so problems due to unclear storage conditions are likely to be relatively minor.
	Metric 7:	Exposure timing, frequency, and duration	Medium	Dosing covered most of the sensitive window for developmental outcomes, but there are minor concerns that dosing began one day after implantation occurs in rats, so only part of the window of sensitivity for implantations was covered by the exposure duration. Additionally, the exposure duration ends before the skeletal system is fully formed so only part of the window of sensitivity for skeletal malformations is included. As most of the windows of sensitivity for these effects were included, this deficiency is unlikely to have a large impact on the results. Otherwise, the exposure frequency was appropriate and appeared to be consistent between groups.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	There are no concerns about the species, strain or sample size of the test animal. The number of doses and spacing of doses are appropriate and it appears that the chosen doses were appropriate for POD determination. Outcome assessment methods are appropriate and are enough to well characterize endpoints of interest.
	Metric 9:	Results presentation	High	Statistical methods are appropriate and described in adequate detail. Full quantitative presentation of the results is included with measures of variance. The litter is the unit of sampling for developmental/reproductive endpoints.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Waterman, S. J., Ambroso, J. L., Keller, L. H., Trimmer, G. W., Nikiforov, A. I., Harris, S. B. (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reproductive Toxicology 13(2):131-136.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weights, food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680201 Linked HERO ID(s): 680201, 679108, 680097, 10750187

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	Test substance name, CASRN, purity and source are reported. Test animal species, strain, sex, parity, source and animal housing conditions (such as number of animals/cage, food and water availability, temperature, humidity and light/dark cycle) were reported. Animal starting age was not reported, and starting animal weight is only reported graphically starting from GD 0 (body weights from the start of the quarantine period after the animals arrived are not reported). Exposure details and endpoint assessment methods were reported in adequate detail.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors stated that animals were assigned to dose groups in the order of mating, which is a non-random method of allocation and the authors do not specify if the allocated animals to one dose group first, or if they assigned mated animals to different groups after the completion of mating. This ambiguity could imply some degree of bias if animals with higher reproductive fitness are disproportionately assigned to one group.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No blinding of exposure groups was described, but most endpoints are objective in nature. For data on fetal malformations, no blinding was described, but an external reviewer verified skeletal findings which may minimize potential impacts of observational bias on the results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	An appropriate negative vehicle control was used and there was no response in the control group. The authors measured several factors that may act as confounders (body weights, food consumption) and the gavage route of exposure prevents potential confounding from palatability issues.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The authors state that no dams died. The authors did not state the initial sample size at the start of dosing in the methods but sample size in tables is reported as a range (22-25) in the results, implying that some animals or litters may be excluded from the analysis. The authors do not explain this omission, but it is unlikely to have a large impact on the results. Most endpoints described in the methods appear in the results, with the exception of uterus weights that were described in the methods but do not appear anywhere in the results. The significance of this omission is relatively minor.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Waterman, S. J., Ambroso, J. L., Keller, L. H., Trimmer, G. W., Nikiforov, A. I., Harris, S. B. (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reproductive Toxicology 13(2):131-136.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weights, food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diisononyl Phthalate- Parent compound
HERO ID:	680201 Linked HERO ID(s): 680201, 679108, 680097, 10750187

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test substance is identified definitively by name and CASRN and the reported purity is sufficient. The authors performed their own independent analytical verification of the test substance purity using an acceptable method. There are no concerns regarding test substance preparation. The gavage volume is acceptable, and the route of exposure is appropriate for the COI. Test substance storage conditions were not described, but the COI has high stability, and the exposure period was relatively short, so problems due to unclear storage conditions are likely to be relatively minor.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure duration and frequency covered the entirety of the sensitive window for endpoints of interest and appeared to be consistent between groups.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	There are no concerns about the species, strain or sample size of the test animal. The number of doses and spacing of doses are appropriate and it appears that the chosen doses were appropriate for POD determination. Outcome assessment methods are appropriate and are sufficient to well characterize endpoints of interest.
	Metric 9:	Results presentation	High	Statistical methods are appropriate and described in adequate detail. Quantitative presentation of the results is included but measures of variance are omitted from figures.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Survivorship, unscheduled deaths.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported for the endpoints/outcome of interest. The chemical name (Di-isononyl phthalate, DINP), CASRN# (68515-48-0), the chemical form was characterized (clear colorless liquid), the purity (100%), the exposure concentration of low (I), med (II), and high (III) and unexposed control, the duration of exposure (6,12,18 and up to 24 month), and the route of exposure (diet) were provided. The test animal species (rat), strain (Fischer-344), sex (both male and female), animal supplier (Charles River Breeding Laboratories), body weights at initiation of dosing were reported (males: 106-143g; females: 88-110g). Animal age at the time of exposure was specified (6 weeks old). Information on animal husbandry; temperature (68-76 F), humidity (68-76% relative humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (animal housed individually except during the first week of acclimation- 19 days acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (440/sex, 110/group) was provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for metric 2.1. Animals were selected using a computer-generated sorting to minimize body weight variation between the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, body weight, hematology, clinical chemistry) and result of initial histopathology review (HERO ID 1325509) upgraded the rank of this domain .
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative control groups was included, and the use of negative control was reported. A positive control is not required based on this study type. No effect of test substance palatability in dietary exposure, differences in test material consumption in certain weeks (study schedule change) of the study may have effect related to differences in food consumption or body weight was reported among the study group. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Survivorship, unscheduled deaths.
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most prespecified outcomes. The study reported the number of the animals (total of 201) died prior to scheduled sacrifice, and the causes of death to specific animal dose groups were specified (HERO ID 132550). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP) and CASRN # (68515-48-0), source and 100% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the stability of the test material and analysis of the concentration of test material in feed was conducted every time was prepared and the test diet presented to the animals. No concern about the test administration in the diet; were fixed weight percent (within 10% of its target concentration) and test animals were divided into 3 groups at 3 dose levels and untreated control. As indicated by the authors, difficulties with analytical methodology variation except for diet analytical and homogeneity were corrected and had no significant impact on the outcome of the study.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The route and duration of exposure were appropriate for the study type and outcomes
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal selected, species, strain sex, life-stage (rat- Fischer-344, 6 weeks old male and female) was relevant to evaluation of the outcomes. Sample size (n=110/sex/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology were addressed.
	Metric 9:	Results presentation	High	Quantitative date were provided. The survivorship analysis was based on two methods. The first method was estimation of median survivorship (Weibull), and the second method was estimating survivorship between groups (Kaplan-Meier using Kruskal-Wallis and Cox's test). It was clearly reported animals that prematurely died.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain/loss, food consumption
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported for the endpoints/outcome of interest. The chemical name (Di-isononyl phthalate, DINP), CASRN# (68515-48-0), the chemical form was characterized (clear colorless liquid), the purity (100%), the exposure concentration of low (I), med (II), and high (III) and unexposed control, the duration of exposure (6,12,18 and up to 24 month), and the route of exposure (diet) were provided. The test animal species (rat), strain (Fischer-344), sex (both male and female), animal supplier (Charles River Breeding Laboratories), body weights at initiation of dosing were reported (males: 106-143g; females: 88-110g). Animal age at the time of exposure was specified (6 weeks old). Information on animal husbandry; temperature (68-76 F), humidity (68-76% relative humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (animal housed individually except during the first week of acclimation- 19 days acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (440/sex, 110/group) was provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for metric 2.1. Animals were selected using a computer-generated sorting to minimize body weight variation between the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, body weight, hematology, clinical chemistry) and result of initial histopathology review (HERO ID 1325509) upgraded the rank of this domain .
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative control groups was included, and the use of negative control was reported. A positive control is not required based on this study type. No effect of test substance palatability in dietary exposure, differences in test material consumption in certain weeks (study schedule change) of the study may have effect related to differences in food consumption or body weight was reported among the study group. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, body weight gain/loss, food consumption
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most prespecified outcomes. The study reported the number of the animals (total of 201) died prior to scheduled sacrifice, and the causes of death to specific animal dose groups were specified (HERO ID 132550). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP) and CASRN # (68515-48-0), source and 100% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the stability of the test material and analysis of the concentration of test material in feed was conducted every time was prepared and the test diet presented to the animals. No concern about the test administration in the diet; were fixed weight percent (within 10% of its target concentration) and test animals were divided into 3 groups at 3 dose levels and untreated control. As indicated by the authors, difficulties with analytical methodology variation except for diet analytical and homogeneity were corrected and had no significant impact on the outcome of the study.
	Metric 7:	Exposure timing, frequency, and duration	High	Test material administered in the diet, the route and duration of exposure were appropriate for the study type and outcomes
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The test animal selected, species, strain sex, life-stage (rat- Fischer-344, 6 weeks old male and female) was relevant to evaluation of the outcomes. Sample size (n=110/sex/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology addressed the proposed outcomes.
	Metric 9:	Results presentation	High	Quantitative date were provided, body weight and food consumption were statistically analyzed for significant differences There was quantitative presentation of results, individual and mean weekly food consumption, body weight by sex and group was calculated and statistically evaluated

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-organ weights (liver), clinical chemistry (Aspartate aminotransferase [AST/GOT]; alanine aminotransferase [ALT/GPT]; alkaline phosphatase [ALP], bilirubin [BIL], total protein [TP], triglycerides, blood urea nitrogen (BUN], albumin, cholesterol [TC]), gross necropsy (liver)-Renal/Kidney-Organ weights (kidney), clinical chemistry (electrolytes (NA, K, Cl), calcium, glucose, creatinine, urinalysis (PH, specific gravity, ketones, bilirubin, occult blood, volume, glucose, protein, creatinine, renal epithelial cell count, sodium, potassium, osmolality), gross necropsy (kidney), urinary bladder.-Other (please specify below) (Endocrine)-Organ weights (adrenals), gross necropsy (adrenals), (pituitary), pancreas, adrenals.-Immune/Hematological-Organ weights (spleen), hematology (erythrocyte count [RBC], hematocrit [HCT], hemoglobin [Hb], leukocyte count [WBC); total and differential, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]), A/G ration, globulin, gross necropsy(spleen), thymus, bone marrow, lymph nodes
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported for the endpoints/outcome of interest. The chemical name (Di-isononyl phthalate, DINP), CASRN# (68515-48-0), the chemical form was characterized (clear colorless liquid), the purity (100%), the exposure concentration of low (I), med (II), and high (III) and unexposed control, the duration of exposure (6,12,18 and up to 24 month), and the route of exposure (diet) were provided. The test animal species (rat), strain (Fischer-344), sex (both male and female), animal supplier (Charles River Breeding Laboratories), body weights at initiation of dosing were reported (males: 106-143g; females: 88-110g). Animal age at the time of exposure was specified (6 weeks old). Information on animal husbandry; temperature (68-76 F), humidity (68-76% relative humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (animal housed individually except during the first week of acclimation- 19 days acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (440/sex, 110/group) was provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for metric 2.1. Animals were selected using a computer-generated sorting to minimize body weight variation between the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, body weight, hematology, clinical chemistry) and result of initial histopathology review (HERO ID 1325509) upgraded the rank of this domain .
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative control groups was included, and the use of negative control was reported. A positive control is not required based on this study type. No effect of test substance palatability in dietary exposure. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-organ weights (liver), clinical chemistry (Aspartate aminotransferase [AST/GOT]; alanine aminotransferase [ALT/GPT]; alkaline phosphatase [ALP], bilirubin [BIL], total protein [TP], triglycerides, blood urea nitrogen (BUN], albumin, cholesterol [TC]), gross necropsy (liver)-Renal/Kidney-Organ weights (kidney), clinical chemistry (electrolytes (NA, K, Cl), calcium, glucose, creatinine, urinalysis (PH, specific gravity, ketones, bilirubin, occult blood, volume, glucose, protein, creatinine, renal epithelial cell count, sodium, potassium, osmolality), gross necropsy (kidney), urinary bladder.-Other (please specify below) (Endocrine)-Organ weights (adrenals), gross necropsy (adrenals), (pituitary), pancreas, adrenals.-Immune/Hematological-Organ weights (spleen), hematology (erythrocyte count [RBC], hematocrit [HCT], hemoglobin [Hb], leukocyte count [WBC]; total and differential, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]), A/G ration, globulin, gross necropsy(spleen), thymus, bone marrow, lymph nodes
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most prespecified outcomes. The study reported the number of the animals (total of 201) died prior to scheduled sacrifice, and the causes of death to specific animal dose groups were specified (HERO ID 132550). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP) and CASRN # (68515-48-0), source and 100% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the stability of the test material and analysis of the concentration of test material in feed was conducted every time was prepared and the test diet presented to the animals. No concern about the test administration in the diet; were fixed weight percent (within 10% of its target concentration) and test animals were divided into 3 groups at 3 dose levels and untreated control. As indicated by the authors, difficulties with analytical methodology variation except for diet analytical and homogeneity were corrected and had no significant impact on the outcome of the study.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study intended to measure the chronic toxicity and oncogenesis effect of DINP when administered in the diet, the route and duration of exposure were appropriate for the study type and outcomes
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The test animal selected, species, strain sex, life-stage (rat- Fischer-344, 6 weeks old male and female) was relevant to evaluation of the outcomes. Sample size (n=110/sex/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology addressed the proposed outcomes (e.g., serum chemistry, hematology, organ weight and necropsy finding evaluated. Minor limitations were identified in the sampling of the outcomes (e.g., nasal cavity tissues were performed for high dose and controls, kidneys, liver were performed in low and mid dose groups sacrificed at study termination).

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-organ weights (liver), clinical chemistry (Aspartate aminotransferase [AST/GOT]; alanine aminotransferase [ALT/GPT]; alkaline phosphatase [ALP], bilirubin [BIL], total protein [TP], triglycerides, blood urea nitrogen (BUN], albumin, cholesterol [TC]), gross necropsy (liver)-Renal/Kidney-Organ weights (kidney), clinical chemistry (electrolytes (NA, K, Cl), calcium, glucose, creatinine, urinalysis (PH, specific gravity, ketones, bilirubin, occult blood, volume, glucose, protein, creatinine, renal epithelial cell count, sodium, potassium, osmolality), gross necropsy (kidney), urinary bladder.-Other (please specify below) (Endocrine)-Organ weights (adrenals), gross necropsy (adrenals), (pituitary), pancreas, adrenals.-Immune/Hematological-Organ weights (spleen), hematology (erythrocyte count [RBC], hematocrit [HCT], hemoglobin [Hb], leukocyte count [WBC); total and differential, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]), A/G ration, globulin, gross necropsy(spleen), thymus, bone marrow, lymph nodes
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Organ weight (liver, kidney, adrenal, spleen) were statistically analyzed for significant differences. The mean organ weights by sex and group for the animals sacrificed at 6-18 months and at study termination have been statistically analyzed and were presented in tables. Individual values for each animal and mean relative organ weights were calculated and presented. There were quantitative blood hematology and biochemistry parameter by sex and group, and qualitive individual values for animals by sex and group sacrificed at 6,12, 18 months, and at study termination and were statistically analyzed and presented.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Cardiovascular-Organ weights (heart), gross necropsy (heart),-Reproductive/Developmental-Organ weights: (ovary, testes), gross necropsy (ovaries, testes), epididymites, seminal vesicles, prostate, mammary glands, uterus, vagina, cervical (females).-Neurological/Behavioral-Organ weights (brain), gross necropsy (brain), nerve, spinal cord)-Thyroid-organ weight (thyroid and parathyroid), gross necropsy (thyroid and parathyroid).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported for the endpoints/outcome of interest. The chemical name (Di-isononyl phthalate, DINP), CASRN# (68515-48-0), the chemical form was characterized (clear colorless liquid), the purity (100%), the exposure concentration of low (I), med (II), and high (III) and unexposed control, the duration of exposure (6,12,18 and up to 24 month), and the route of exposure (diet) were provided. The test animal species (rat), strain (Fischer-344), sex (both male and female), animal supplier (Charles River Breeding Laboratories), body weights at initiation of dosing were reported (males: 106-143g; females: 88-110g). Animal age at the time of exposure was specified (6 weeks old). Information on animal husbandry; temperature (68-76 F), humidity (68-76% relative humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (animal housed individually except during the first week of acclimation- 19 days acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (440/sex, 110/group) was provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for metric 2.1. Animals were selected using a computer-generated sorting to minimize body weight variation between the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature and result of initial histopathology review (HERO ID 1325509) upgraded the rank of this domain .
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative control groups was included, and the use of negative control was reported. A positive control is not required based on this study type. No effect of test substance palatability in dietary exposure. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most prespecified outcomes. The study reported the number of the animals died prior to scheduled sacrifice, and the causes of death to specific animal dose groups were specified (HERO ID 132550). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Cardiovascular-Organ weights (heart), gross necropsy (heart),-Reproductive/Developmental-Organ weights: (ovary, testes), gross necropsy (ovaries, testes), epididymites, seminal vesicles, prostate, mammary glands, uterus, vagina, cervical (females).-Neurological/Behavioral-Organ weights (brain), gross necropsy (brain), nerve, spinal cord)-Thyroid-organ weight (thyroid and parathyroid), gross necropsy (thyroid and parathyroid).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP) and CASRN # (68515-48-0), source and 100% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the stability of the test material and analysis of the concentration of test material in feed was conducted every time was prepared and the test diet presented to the animals. No concern about the test administration in the diet; were fixed weight percent (within 10% of its target concentration) and test animals were divided into 3 groups at 3 dose levels and untreated control. As indicated by the authors, difficulties with analytical methodology variation except for diet analytical and homogeneity were corrected and had no significant impact on the outcome of the study.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study intended to measure the chronic toxicity and oncogenesis effect of DINP when administered in the diet, the route and duration of exposure were appropriate for the study type and outcomes
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The test animal selected, species, strain sex, life-stage (rat- Fischer-344, 6 weeks old male and female) was relevant to evaluation of the outcomes. Sample size (n=110/sex/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology addressed the proposed outcomes (e.g., organ weight and necropsy finding evaluated).
	Metric 9:	Results presentation	High	Organ weight (heart, brain, ovaries and testes, and thyroid/parathyroid) were statistically analyzed for significant differences. The mean organ weights by sex and group for the animals sacrificed at 6-18 months and at study termination have been statistically analyzed and were presented in tables. Individual values for each animal and mean relative organ weights were calculated and presented.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (clinical observation)-Poor conditions, general observation within normal limits, emaciated, alopecia, scabbing, ocular opacity, enlarged, vascularization, and clear/red ocular discharge, swollen around eye, eye closed/partially closed pinworms in the anal region, red discharge anus, pale extremities or pale-yellow extremities, urinary staining, little sign of stool, soft stool, fecal staining, malocclusion and broken incisor, mass, nasal and dried red nasal discharge, dyspnea, sores, wet and dry rales, swollen ventral head, head leans, bump on tail, kink in tail, necrotic tail, protruding penis, red discharge penis, red material snout, red material seen an out/around eye(s), abdominal griping, swollen abdomen, blue testes, swollen around the eye/anus/testes/ventral cervical, hypothermia, hypoactivity, prostration, impaired use hindleg, red vaginal discharge.-Lung/Respiratory-Organ weights (lung, trachea), gross necropsy, nasal turbinates/cavity, trachea, larynx, lungs, pharynx-Skin/Connective Tissue-Skin, subcutaneous palpable masses, icterus of subcutaneous tissues-Ocular/Sensory-Ocular opacity, vascularization, and discharge, ophthalmoscopic (eye with optic nerve, harderian gland).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported for the endpoints/outcome of interest. The chemical name (Di-isononyl phthalate, DINP), CASRN# (68515-48-0), the chemical form was characterized (clear colorless liquid), the purity (100%), the exposure concentration of low (I), med (II), and high (III) and unexposed control, the duration of exposure (6,12,18 and up to 24 month), and the route of exposure (diet) were provided. The test animal species (rat), strain (Fischer-344), sex (both male and female), animal supplier (Charles River Breeding Laboratories), body weights at initiation of dosing were reported (males: 106-143g; females: 88-110g). Animal age at the time of exposure was specified (6 weeks old). Information on animal husbandry; temperature (68-76 F), humidity (68-76% relative humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (animal housed individually except during the first week of acclimation- 19 days acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (440/sex, 110/group) was provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for metric 2.1. Animals were selected using a computer-generated sorting to minimize body weight variation between the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, body weight, hematology, clinical chemistry) and result of initial histopathology review (HERO ID 1325509) upgraded the rank of this domain .
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative control groups was included, and the use of negative control was reported. A positive control is not required based on this study type. No effect of test substance palatability in dietary exposure. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (clinical observation)-Poor conditions, general observation within normal limits, emaciated, alopecia, scabbing, ocular opacity, enlarged, vascularization, and clear/red ocular discharge, swollen around eye, eye closed/partially closed pinworms in the anal region, red discharge anus, pale extremities or pale-yellow extremities, urinary staining, little sign of stool, soft stool, fecal staining, malocclusion and broken incisor, mass, nasal and dried red nasal discharge, dyspnea, sores, wet and dry rales, swollen ventral head, head leans, bump on tail, kink in tail, necrotic tail, protruding penis, red discharge penis, red material snout, red material seen an out/around eye(s), abdominal griping, swollen abdomen, blue testes, swollen around the eye/anus/testes/ventral cervical, hypothermia, hypoactivity, prostration, impaired use hindleg, red vaginal discharge.-Lung/Respiratory-Organ weights (lung, trachea), gross necropsy, nasal turbinates/cavity, trachea, larynx, lungs, pharynx-Skin/Connective Tissue-Skin, subcutaneous palpable masses, icterus of subcutaneous tissues-Ocular/Sensory-Ocular opacity, vascularization, and discharge, ophthalmoscopic (eye with optic nerve, harderian gland).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most prespecified outcomes. The study reported the number of the animals (total of 201) died prior to scheduled sacrifice, and the causes of death to specific animal dose groups were specified (HERO ID 132550). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP) and CASRN # (68515-48-0), source and 100% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the stability of the test material and analysis of the concentration of test material in feed was conducted every time was prepared and the test diet presented to the animals. No concern about the test administration in the diet; were fixed weight percent (within 10% of its target concentration) and test animals were divided into 3 groups at 3 dose levels and untreated control. As indicated by the authors, difficulties with analytical methodology variation except for diet analytical and homogeneity were corrected and had no significant impact on the outcome of the study.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study intended to measure the chronic toxicity and oncogenesis effect of DINP when administered in the diet, the route and duration of exposure were appropriate for the study type and outcomes
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The test animal selected, species, strain sex, life-stage (rat- Fischer-344, 6 weeks old male and female) was relevant to evaluation of the outcomes. Sample size (n=110/sex/group) and the timing of the endpoint assessment was suitable. The outcome assessment methodology addressed the proposed outcomes (e.g., serum chemistry, hematology, organ weight and necropsy finding evaluated. Minor limitations were identified in the sampling of the outcomes (e.g., nasal cavity tissues were performed for high dose and controls, kidneys, liver were performed in low and mid dose groups sacrificed at study termination).
	Metric 9:	Results presentation	Medium	Incidence of clinical and in-life observations for all and individual survived animal results were were reported

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (clinical observation)-Poor conditions, general observation within normal limits, emaciated, alopecia, scabbing, ocular opacity, enlarged, vascularization, and clear/red ocular discharge, swollen around eye, eye closed/partially closed pinworms in the anal region, red discharge anus, pale extremities or pale-yellow extremities, urinary staining, little sign of stool, soft stool, fecal staining, malocclusion and broken incisor, mass, nasal and dried red nasal discharge, dyspnea, sores, wet and dry rales, swollen ventral head, head leans, bump on tail, kink in tail, necrotic tail, protruding penis, red discharge penis, red material snout, red material seen an out/around eye(s), abdominal griping, swollen abdomen, blue testes, swollen around the eye/anus/testes/ventral cervical, hypothermia, hypoactivity, prostration, impaired use hindleg, red vaginal discharge.-Lung/Respiratory-Organ weights (lung, trachea), gross necropsy, nasal turbinates/cavity, trachea, larynx, lungs, pharynx-Skin/Connective Tissue-Skin, subcutaneous palpable masses, icterus of subcutaneous tissues-Ocular/Sensory-Ocular opacity, vascularization, and discharge, ophthalmoscopic (eye with optic nerve, harderian gland).
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric	Rating	Comments

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Gastrointestinal-Gross necropsy (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, small intestine, rectum, salivary glands)-Musculoskeletal-Gross necropsy, aorta, femoris muscle with sciatic, midthoracic, lumbar, sternum with marrow bone, skeletal muscle)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported for the endpoints/outcome of interest. The chemical name (Di-isononyl phthalate, DINP), CASRN# (68515-48-0), the chemical form was characterized (clear colorless liquid), the purity (100%), the exposure concentration of low (I), med (II), and high (III) and unexposed control, the duration of exposure (6,12,18 and up to 24 month), and the route of exposure (diet) were provided. The test animal species (rat), strain (Fischer-344), sex (both male and female), animal supplier (Charles River Breeding Laboratories), body weights at initiation of dosing were reported (males: 106-143g; females: 88-110g). Animal age at the time of exposure was specified (6 weeks old). Information on animal husbandry; temperature (68-76 F), humidity (68-76% relative humidity), and 12 hours light/dark cycle were reported. The number of animals per cage (animal housed individually except during the first week of acclimation- 19 days acclimation), diet and water availability were clearly reported. The endpoint evaluation methods were described, and quantitative results were reported endpoint. Sample size (440/sex, 110/group) was provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for metric 2.1. Animals were selected using a computer-generated sorting to minimize body weight variation between the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	The study is considered high for Metric 2.2. Blinding to reduce observational bias were not reported; however, the endpoints evaluated were either not subjective in nature (e.g., mortality, body weight, hematology, clinical chemistry) and result of initial histopathology review (HERO ID 1325509) upgraded the rank of this domain .
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative control groups was included, and the use of negative control was reported. A positive control is not required based on this study type. No effect of test substance palatability in dietary exposure. All animal husbandry conditions: temperature, humidity, light/dark cycle, diet, water availability, ad libitum, number of animals per cage were adequate and consistent across study groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most prespecified outcomes. The study reported the number of the animals (total of 201) died prior to scheduled sacrifice, and the causes of death to specific animal dose groups were specified (HERO ID 132550). Overall, the attrition was explained therefore it has no significant impact on the interpretation of the results

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Study Citation:	Bio/dynamics, (1986). Chronic toxicity/oncogenicity study in F-344 rats (final report) with cover letter dated 042386.
Health Outcome(s) and Reported Health Effect(s):	Gastrointestinal-Gross necropsy (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, small intestine, rectum, salivary glands)-Musculoskeletal-Gross necropsy, aorta, femoris muscle with sciatic, midthoracic, lumbar, sternum with marrow bone, skeletal muscle)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-6-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-12-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-18-month(s)-Oral-Diet-Duration: Chronic (>90 days)-7-24-2-year(s)
Species:	Rat-Fischer 344 - [rat]-Both
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	1065989 Linked HERO ID(s): 1065989, 1239588, 1325509

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance was identified by name (DINP) and CASRN # (68515-48-0), source and 100% purity were reported. However, there was no independent analytical verification of the test article purity performed. The authors indicated the stability of the test material and analysis of the concentration of test material in feed was conducted every time was prepared and the test diet presented to the animals. No concern about the test administration in the diet; were fixed weight percent (within 10% of its target concentration) and test animals were divided into 3 groups at 3 dose levels and untreated control. As indicated by the authors, difficulties with analytical methodology variation except for diet analytical and homogeneity were corrected and had no significant impact on the outcome of the study.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study intended to measure the chronic toxicity and oncogenesis effect of DINP when administered in the diet, the route and duration of exposure were appropriate for the study type and outcomes
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	No results were displayed
	Metric 9:	Results presentation	Uninformative	No results were presented

Additional Comments: None

Overall Quality Determination Uninformative

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 Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
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.
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 Control				
	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, plastic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophosphates. No analysis of food for potential endocrine disruptors was conducted.

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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 Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	788239

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The text states that there was no mortality, overt toxicity, or reduced maternal body weight or reduced litter size at any of the tested doses, indicating no attrition for other endpoints The methods state that there were 3-6 dams group treated with DINP CASRN 28553-12-0 and 3/group treated with CASRN 68515-48-0. There were no dam mortalities. Data from both CASRNs were combined in Table 1; the sample sizes for two dose groups (500 and 750 mg/kg-day), were n=5 litters. Based on the dams treated/group, a minimum of 6 litters was expected. It is unclear whether there was any unreported animal attrition, or if this represents an outlier, or selective reporting. No author justification was provided.
Domain 5: Exposure Methods Sensitivity				
	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. Sigma's website reports this chemical as ester content as $\geq$99.5% (mixture of C9 isomers), technical grade, with impurities of $\leq$0.15% dioctyl phthalate. The test substance was not analytically verified by the performing laboratory. The study did not measure concentration in corn oil or report if doses were prepared fresh. Preparation (e.g., homogeneity) and storage details were not provided. Dams were dosed daily by gavage It is not reported whether doses were adjusted daily based on maternal body weight.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. Pregnant 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 Measures and Results Display				

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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 Health Effect(s):	Reproductive/Developmental-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	788239

Domain	Metric		Rating	Comments
	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, which may have reduced the accuracy of the results. 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 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. Overall, the sample size was small (n=3 dams/dose group), which may reduce the sensitivity or statistical power.
	Metric 9:	Results presentation	Low	Data for DINP (CAS RN 28553-12-0) and DINP (mixed isomers; CAS RN 68515-48-0) were not significantly different, therefore study authors combined the data from the two chemicals in Table 1 (mean testosterone production (with SE). The study does report data on the two chemicals independently in Figure 6. This figure shows testosterone production as percentage of control, and supports the authors claim that responses from the two chemicals were similar. Figure 6 does not report the number of animals for each chemical; Table 1 reports a combined number of animals.

Additional Comments: None

Overall Quality Determination 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 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):	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
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.
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 Control				
	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, plastic bottles could leach phthalates that could confound results. Municipal water was analyzed for contaminates, but it is not known if this included analysis for organophosphates. No analysis of food for potential endocrine disruptors was conducted.
Domain 4: Selective Reporting and Attrition				

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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 Health Effect(s):	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The text states that there was no mortality, overt toxicity, or reduced maternal body weight or reduced litter size at any of the tested doses, indicating no attrition for other endpoints. The methods state that there were 3-6 dams group treated with DINP CASRN 28553-12-0 and 3/group treated with CASRN 68515-48-0. There were no dam mortalities. Data from both CASRNs were combined in Table 1; the sample sizes for two dose groups (500 and 750 mg/kg-day), were n=5 litters. Based on the dams treated/group, a minimum of 6 litters was expected. It is unclear whether there was any unreported animal attrition, or if this represents an outlier, or selective reporting. No author justification was provided.
Domain 5: Exposure Methods Sensitivity				
	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, however Sigma's website reports purity ester content as >=99.5% (mixture of C9 isomers). The study did not measure concentration in corn oil or report if doses were prepared fresh. Dams were dosed daily by oral gavage between 8-10am each day. It is not reported whether doses were adjusted daily based on maternal body weight.
	Metric 7:	Exposure timing, frequency, and duration	Medium	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 Measures and Results 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 publication 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 adequate to consistently detect anything other than rather large alterations of maternal weight gain. Clinical signs: The authors did not report how often animals were assessed for clinical signs of toxicity.
	Metric 9:	Results presentation	Medium	Data were reported as negative in the text.

Additional Comments: None

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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 Health Effect(s):	Reproductive/Developmental-Developmental -litter size; fetal mortality-Nutritional/Metabolic-Maternal body weight and body weight gain-Other (please specify below) (Clinical signs)-Overt toxicity (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	788239

Domain	Metric	Rating	Comments
Overall Quality Determination		**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 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)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
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.
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.
	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 Control				
	Metric 4:	Confounding / Variable Control	Medium	The negative control group was included and appropriate. There were no indication husbandry conditions were different between the groups. There is no indication of infection or any other health condition occurred in the animals. Food and water intake were not reported. 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 substantially 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 results.
Domain 4: Selective Reporting and Attrition				

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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 Health Effect(s):	Mortality-Mortality (results reported for DINP and DIBP only)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Harlan)-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	The text states that there was no mortality, overt toxicity, or reduced maternal body weight or reduced litter size at any of the tested doses, indicating no attrition for other endpoints. The number of pregnant dams reported in the methods is in agreement with the numbers assessed (as reported in Table 1).
Domain 5: Exposure Methods Sensitivity				
	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, however Sigma's website reports purity ester content as >=99.5% (mixture of C9 isomers). The study did not measure concentration in corn oil or report if doses were prepared fresh. Dams were dosed daily by oral gavage between 8-10am each day. It is not reported whether doses were adjusted daily based on maternal body weight.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. Pregnant 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 Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The study did not report how often animals were assessed for mortality; however, no deaths occurred. The lack of this information does not affect the ability to assess this endpoint. The animal model was appropriate for the outcome of interest. The sample size was small (n= 3 dams/group), but this is not expected to have a significant impact on this outcome.
	Metric 9:	Results presentation	Medium	Data were reported as negative in the text.

Additional Comments: None

Overall Quality Determination — Medium

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):	Mortality-Maternal lethality
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were reported. The test material was clearly identified by name and CASRN. The purity was reported along with the general composition. The source was not explicitly stated (commercial source). 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 Attrition				
	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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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):	Mortality-Maternal lethality
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked 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-dimethylheptanol-1. The nominal doses were calculated based on animal body weights at the beginning 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 Measures and Results 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 animals/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 Quality Determination Medium

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):	Reproductive/Developmental-Reproductive: Uterus weight, corpora lutia/dam, implantations sites/dam, placental weight; Developmental: pre and post implantation loss, total resorptions, live fetuses, fetal weights, fetal and skeletal variations and malformations
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were reported. The test material was clearly identified by name and CASRN. The purity was reported along with the general composition. The source was not explicitly stated (commercial source). 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 Attrition				
	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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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):	Reproductive/Developmental-Reproductive: Uterus weight, corpora lutia/dam, implantations sites/dam, placental weight; Developmental: pre and post implantation loss, total resorptions, live fetuses, fetal weights, fetal and skeletal variations and malformations
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked 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 $\geq$99%. The alcohol moiety consisted of equivalent amounts of 3,4-, 4,6-, 3,5-, 4,5-, and 5,6-dimethylheptanol-1. The nominal doses were calculated based on animal body weights at the beginning 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 Measures and Results 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 variance. 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 Quality Determination Medium

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):	Other (please specify below) (Clinical signs)-Maternal clinical signs-Nutritional/Metabolic-Maternal body weights, food consumption, body weight change
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were reported. The test material was clearly identified by name and CASRN. The purity was reported along with the general composition. The source was not explicitly stated (commercial source). 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 inconsistent. No other confounding variables were reported.
Domain 4: Selective Reporting and Attrition				
	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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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):	Other (please specify below) (Clinical signs)-Maternal clinical signs-Nutritional/Metabolic-Maternal body weights, food consumption, body weight change
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-15)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked 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-heptanol-1. The nominal doses were calculated based on animal body weights at the beginning 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, duration, and frequency were adequate for the selected outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	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 those specified in OECD TG 414.
	Metric 9:	Results presentation	Low	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 were appropriate.

Additional Comments: None

Overall Quality Determination Medium

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:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were reported. The test material was clearly identified by name and CASRN. The purity was reported along with the general composition. The source was not explicitly stated (commercial source). 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 Performance				
	Metric 2:	Allocation	Low	No details describing the method of animal allocation or other indicators of normalization 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 Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent sham-treated control group was included; the study used an olive oil vehicle. 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 Attrition				
	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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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:	Diisononyl Phthalate- Isomer: Di-isononyl phthalate (mixed isomers) - CASRN 68515-48-0
HERO ID:	674193 Linked 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-heptanol-1. The nominal doses were calculated based on animal body weights at the beginning 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, duration, and frequency were adequate for the selected outcomes of interest.
Domain 6: Outcome Measures and Results 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 partially sensitive to the outcomes of interest; only organ weights were measured. Clinical chemistry and microscopic analysis were not included.
	Metric 9:	Results presentation	Low	Organ weight data were presented as means without measures of variance. Statistical methods were described and were appropriate.

Additional Comments: None

Overall Quality Determination Medium