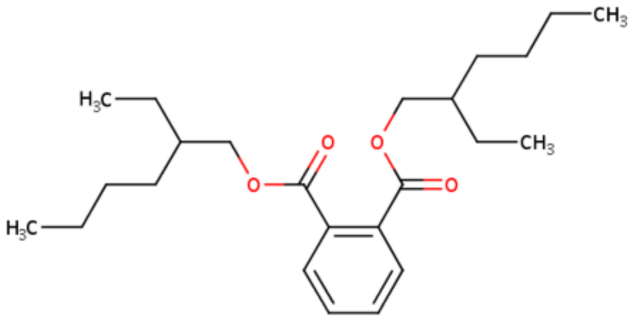
United States
Environmental Protection Agency

May 2025
Office of Chemical Safety and
Pollution Prevention

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

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 117-81-7



May 2025

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

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Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study does 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 or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for all prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.
Domain 5: Exposure Methods Sensitivity				

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Results for most endpoints were described in the text and data were presented in tables as means ± standard error. Statistical tests were reported and appropriate. Histopathological data were reported as negative in the text.

Additional Comments: Study included 3 different durations of exposure during post weaning up to adulthood. each ”experiment” included different outcomes and were potentially performed on different cohorts of animals (though this is unclear and all groups were >10). not all outcomes were evaluated for each duration. This evaluation corresponds with the PND21-48 duration

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproductive effects. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data). Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means ± SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproductive effects. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data). Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means ± SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.

Additional Comments: None

Overall Quality Determination — Medium

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported; qualitative results were reported for some, but not all outcomes.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, organ weights)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were qualitatively reported for some reproductive outcomes in the text. Organ weight results were not reported. The text specified the number of animals included in each group, it is not clear whether the sample sizes were equal to the number of animals per group.
Domain 5: Exposure Methods Sensitivity				

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during PND 62-89 which is the period relevant for male reproductive tract development. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focuses on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not specified. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Low	Data were not shown for any endpoint. Results were only reported in the text stating that no effects were reportedly observed; however, no results for organ weights were described in the text. Statistical analysis methods were reported.

Additional Comments: None

Overall Quality Determination — Medium

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for all outcomes in text or table. The number of animals per group was reported and consistent and all animals were used to assess each endpoint. There was no indication of selective reporting/attrition.
Domain 5: Exposure Methods Sensitivity				

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproductive effects. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means ± SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. These missing details are not expected to have a significant impact on the selected endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproductive effects. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data), but were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means ± SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. These missing details are not expected to have a significant impact on the selected endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes in text or table except for maternal food intake. This is not expected to significantly impact the study results. The number of animals per group was reported and consistent; sample sizes were reported for most outcomes and suggest that no animals died. For endpoints where sample sizes were not specified, there is insufficient information to determine whether there was selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is relevant for assessing male reproductive effects. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not reported in Table 2 (PND21-34 experiment), or in Table 4 (PND 35-48 data), but were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Medium	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs (with standard error bars). Histopathology results were only reported in the text and no effects were reportedly observed. Results were reported in tables for body weights (shown as means ± SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported; qualitative results were reported for some, but not all outcomes.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, organ weights)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. These missing details are not expected to have a significant impact on the interpretation of the results for these endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were qualitatively reported for some reproductive outcomes in the text. Organ weight results were not reported. The text specified the number of animals included in each group, it is not clear whether the sample sizes were equal to the number of animals per group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during PND 62-89 which is the period relevant for male reproductive tract development. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focuses on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were not specified. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Low	Data were not shown for any endpoint. Results were only reported in the text stating that no effects were reportedly observed; however, no results for organ weights were described in the text. Statistical analysis methods were reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Initial body weight was reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of animals/group was reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature or did not require blinding (e.g., hormone concentrations, histology, body and organ weights, food consumption)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint of interest (e.g., body weight)
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for all outcomes in text or table. The number of animals per group was reported and consistent and all animals were used to assess each endpoint. There was no indication of selective reporting/attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the prepubertal period and is appropriate for the endpoints of interest (e.g., body weights)
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 421 and 422 Guidelines which focus on reproductive toxicity recommend using at least 10 males which the study did. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups was adequate (there were 4 treated groups). Outcome methodologies for the animals were adequately reported and sensitive for the endpoints assessed. The methods did not specify the timing of body weight and feed intake measurements, but the results noted body weights were measured at the beginning and end of the exposure. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Medium	Results were reported in tables for body weights (shown as means ± SEM). Food intake results were only reported in the text and no effects were reportedly observed. Statistical analysis methods were reported and statistical significance was noted in tables.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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]-Both
Chemical:	Diethylhexyl 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]-Both
Chemical:	Diethylhexyl 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:	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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	7978479

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	The model system used in the paper, CD-1 female mice, was appropriate for the 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:	Diethylhexyl 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 (DEHP) was purchased from Sigma Aldrich (St. Louis, MO), however the CASN was not provided nor was the catalog number from Sigma Aldrich. The study doses animals orally via insertion of a pipette tip into the mouth, utilizes a control (corn oil vehicle), and includes a large range of doses: 20 μg/kg/day, 200 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. 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:	Diethylhexyl 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	Medium	The chemical of interest was purchased from Sigma Aldrich (St. Louis, MO), however, neither the CASN number nor the catalog number from Sigma was indicated leaving room for speculation on the chemicals purity and composition. In a reference from the same author (HERO: 3070927) the purity of DEHP was stated to be 99%. There was no independent verification of the test substance purity, nor were there measures taken to ensure that each animal was getting their full dose. The exposure volume was determined based on body weight taken that day, indicating variable dosing volumes were possible.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The study doses animals orally via insertion of a pipette tip into the mouth, utilizes a control (corn oil vehicle), and includes a large range of doses: 20 μg/kg/day, 200 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. 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:	Diethylhexyl 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 DEHP. 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 percent femalepups from the 12-month post-dosing group for 200 mg/kg/day DEHP (n = 2 mice) was insufficient to perform statistical analysis. Some of the other groups for DEHP have sample sizes of 3, which is quite low. The measurement of sex hormones in sera of female mice was conducted using commercially available enzyme-linked immunosorbent assays (ELISAs) or radioimmunoassays conducted by the University of Virginia Center for Research in Reproduction Ligand Assay and 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.
	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.

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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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	7978481

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl-transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	Critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) was reported; however, no additional details were provided. Neither the number of animals per group or the sample size was specified.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group fed diets in the absence of test substance; however, it does not appear that control animals were included during the post-exposure period. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	Insufficient information was provided to determined attrition or selective reporting.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Uninformative	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake nor body weights were measured and reliable doses in mg/kg-day cannot be determined.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure timing and frequency were reported. Animals were exposed via the diet for 2 weeks. No justification was provided by the study author.

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl-transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	The study included a single endpoint (measurement of hepatic enzyme activities). It is unclear what the purpose of the study was. Enzyme activities alone are not considered to be a sensitive endpoint for assessing hepatic toxicity. No methodological details were provided, although similar measurements were reported for another experiment reported in the same study. Methods of enzyme measurements were cited to other sources.
	Metric 9:	Results presentation	Uninformative	Results were qualitatively described in the study text. An increase in enzyme activity was mentioned; however, no statistical analysis was done and no data were provided to conduct an independent analysis.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testosterone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); the CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	High	The study reports “observers were blinded to treatment condition to avoid bias”. It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testosterone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for most outcomes in tabular form and in the text. The study states that the experiment was ran twice with 10 males/group each time. Data for both trials were combined; therefore there should be data for 20 males/group. Table 1 reports the number of animals as n= 40 (control group); n=19 (for the 10 and 500 mg/kg/day groups); and n=25 (for the 750 mg/kg/day group). It is unclear where the extra animals came from. The authors did not report death, so it is not clear if animals in the 10 and 500 mg/kg/day group died or were not included in analysis for some other reason. Seminal vesicle weights in the 750 mg/kg/day group were not determined; no explanation for this is given.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if study authors adjusted for weight changes when delivering the test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest. The authors state “This age interval was used because the prepubertal period is a time of active reproductive tract development, and hormonally active chemicals are known to exhibit greater potency during sexual differentiation in rodents and humans than at later times.”
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± SEM. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testosterone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); A CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Low	The study reports that “observers were blinded to treatment condition to avoid bias”. It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for all outcomes in tabular form and in the text. The methods do not report how many animals were exposed/group; but based on the 28-day experiment reported in this paper, it is reasonable to assume it may be 10/group. Data were reported for 10 animals/group. The methods specify that rats were exposed to the 500 mg/kg/day dose only for 14 days; however, in the Results (Table 2), data is presented for 10 mg/kg/day and 500 mg/kg/day. It is unclear if the 10 mg/kg/day animals were exposed concurrently or if these data came from an earlier experiment discussed by the authors.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testes, seminal vesicles, and prostate), timing of preputial separation, serum luteinizing hormone and testosterone levels, mRNA expression in pituitary for LH b subunit androgen receptor; testosterone production by isolated Leydig cells in vitro.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if the study authors adjusted for weight changes when delivering the test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest. The study authors wanted to determine whether "compensatory changes in androgen synthesis and feedback suppression of pituitary function were associated with 28-day exposure, so a shorter duration (14-days) was assessed.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± SEM. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); the CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	High	The study reports “observers were blinded to treatment condition to avoid bias”. It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, but this is not expected to impact the ability to interpret the study results for the selected endpoints. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for most outcomes in tabular form and in the text. The study states that the experiment was ran twice with 10 males/group each time. Data for both trials were combined; therefore there should be data for 20 males/group. Table 1 reports the number of animals as n= 40 (control group); n=19 (for the 10 and 500 mg/kg/day groups); and n=25 (for the 750 mg/kg/day group). It is unclear where the extra animals came from. The authors did not report death, so it is not clear if animals in the 10 and 500 mg/kg/day group died or were not included in analysis for some other reason. Seminal vesicle weights in the 750 mg/kg/day group were not determined; no explanation for this is given.

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-28-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if study authors adjusted for weight changes when delivering the test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest. The authors state "This age interval was used because the prepubertal period is a time of active reproductive tract development, and hormonally active chemicals are known to exhibit greater potency during sexual differentiation in rodents and humans than at later times."
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± SEM. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]); A CASRN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, age, and source were reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Low	The study reports that “observers were blinded to treatment condition to avoid bias”. It is unclear if this only applied to assessing preputial separation or all other non-subjective endpoints.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, but this is not expected to impact the ability to interpret the study results for the selected endpoints. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	Data were reported for all outcomes in tabular form and in the text. The methods do not report how many animals were exposed/group; but based on the 28-day experiment reported in this paper, it is reasonable to assume it may be 10/group. Data were reported for 10 animals/group. The methods specify that rats were exposed to the 500 mg/kg/day dose only for 14 days; however, in the Results (Table 2), data is presented for 10 mg/kg/day and 500 mg/kg/day. It is unclear if the 10 mg/kg/day animals were exposed concurrently or if these data came from an earlier experiment discussed by the authors.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Ge, R. S., Chen, G. R., Dong, Q., Akingbemi, B., Sottas, C. M., Santos, M., Sealfon, S. C., Bernard, D. J., Hardy, M. P. (2007). Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. Journal of Andrology 28(4):513-520.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-14-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674162

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. The study does not report information on the storage or preparation of the test substance. Only target concentrations are provided. It is unclear how often/ or if the study authors adjusted for weight changes when delivering the test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest. The study authors wanted to determine whether "compensatory changes in androgen synthesis and feedback suppression of pituitary function were associated with 28-day exposure, so a shorter duration (14-days) was assessed.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses were selected based on previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± SEM. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed.In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal opening to detect first day of estrus.-Nutritional/Metabolic-Body weight of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed.In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal opening to detect first day of estrus.-Nutritional/Metabolic-Body weight of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined. Endpoints examined were sensitive to assess developmental, body weight and clinical sign endpoints.

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed.In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal opening to detect first day of estrus.-Nutritional/Metabolic-Body weight of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	This study is considered High for Metric 6.2. Results were fully reported. Data was presented as means +/- variance and number of animal or litters examined. Statistical analysis was appropriate. Normality and homogeneity of variances were evaluated prior to data analysis. The study used a linear mixed model (proc mixed) with treatment as a main effect and litter as a random factor (nested for treatment) to adjust for litter effects. Organ weights and AGD were analyzed with body weight as a covariate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed.In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal opening to detect first day of estrus.-Nutritional/Metabolic-Body weight of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed.In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal opening to detect first day of estrus.-Nutritional/Metabolic-Body weight of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined. Endpoints examined were sensitive to assess developmental, body weight and clinical sign endpoints.

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Ovary weightLitter size, sex ratio, pup weight, post implantation loss and number of viable pups were assessed.In offspring: clinical signs, were assessed; on PND1 brain and liver weights measured (1-2 females/litter); PND13, all female pups were examined for the number of nipples/areolas. On PND 22, measurement of anogenital distance (AGD) and brain and liver weights. Beginning on PND33, all remaining females were evaluated daily for vaginal opening. Body weights were measured on day of vaginal opening. Daily vaginal smears were assessed from the day of vaginal opening to detect first day of estrus.-Nutritional/Metabolic-Body weight of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	This study is considered High for Metric 6.2. Results were fully reported. Data was presented as means +/- variance and number of animal or litters examined. Statistical analysis was appropriate. Normality and homogeneity of variances were evaluated prior to data analysis. The study used a linear mixed model (proc mixed) with treatment as a main effect and litter as a random factor (nested for treatment) to adjust for litter effects. Organ weights and AGD were analyzed with body weight as a covariate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.

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Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.

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Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
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Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.

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Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
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Species:	Rat-Wistar - [rat]-Female
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Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined for developmental endpoints. Effects on organ systems were assessed by determining changes in organ weights; histopathology was not performed. Although no changes in organ weights were observed, histological evaluation would have provided more information.

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral-Brain weight-Hepatic/Liver-Liver weight-Renal/Kidney-Kidney weight-Thyroid-Thyroid weight-Immune/Hematological-Spleen and thymus weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	This study is considered High for Metric 6.2. Absolute organ weights were reported with variance and n. Relative organ weights were not reported. Although there was no difference in body weights or organ weight, reporting relative weights would be useful. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	This study is considered Medium for Metric 1. Test chemical was described as DEHP, along with lot number and source (Sigma-Aldrich, Germany). Purity was not reported. Dose levels tested, frequency, and route of exposure were reported. Species, strain, sex, source, and initial body weight of animals was reported. Age of the animals was not reported. Husbandry conditions were adequately reported (temperature, humidity, light/dark cycle, food and water). Each animal was individually housed (when not mating). Endpoints evaluated are clearly reported and quantitative data are presented. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	This study is considered Medium for Metric 2.1. Gravid females were randomly assigned to treatment groups, but the study did not describe the specific procedure used. Pups examined at different timepoints were randomly selected for analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	This study is considered medium for Metric 2.2. Anogenital distance and number of nipples/areolas were assessed blindly. Blinding or other measures to reduce observational bias were not reported for the other endpoints evaluated, but lack of blinding is not expected to have a substantial impact on results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	This study is considered Medium for Metric 3. A concurrent appropriate negative control was included. Body weights were similar between groups indicating exposure did not significantly affect food intake (although not explicitly stated). The study did not report infections, although no clinical signs of toxicity were reported. The study did not report all information to determine confounding but the impact on results is expected to be minimal and reported information did not identify differences among study groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	This study is considered Low for Metric 4. The study does not report the number of dams exposed/group in methods, rather the number of dams/group was reported in the results. The number of dams reported/group vary from n=11-16 in results. It is unclear if more dams than the ones reported in the results were exposed and died, or if presumed pregnant dams were in fact not pregnant and therefore not included in analysis. Also, no information was provided as to number pups that may (or may not) have died during lactation, although sample size was reported in results for offspring. Some data points in the Tables indicate a different number of animals were evaluated for different endpoints (for example thyroid weight for dams at 0.405 mg/kg/day n= 14, whereas the other organ weights had n=15). Given the unexplained variation in sample size across the reported outcomes and treatment groups, and the lack of explanation, it is difficult to determine if all animals were included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	This study is considered Low for Metric 5.1. The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. Concentration of all doses was verifiedby gas chromatography/mass spectrometry (cited in HERO 673567). The gavage volume was reported and appropriate (5.0 ml/kg body weight). Although DEHP is non-volatile, lack of details on preparation and storage conditions (i.e., plastic or glass storage bottles or made fresh daily) adds uncertainty about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5. Exposure timing, frequency and duration were appropriate for the study design. Pregnant rats were exposed from GD6-PND21, which is appropriated for the developmental endpoints evaluated. Exposure timing was also appropriate for other endpoints examined given the aim of the study.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered High for Metric 6.1. The number of exposure groups/doses were appropriate, and authors gave justification for doses chosen (based on median daily intake of general German population and doses previously shown to induce adverse effects in male offspring). A NOAEL and LOAEL were determined. Details regarding assessment of clinical signs are lacking. Animals were assessed daily, however, characteristics or behavior the authors would have considered adverse were not provided.

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Study Citation:	Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicological Sciences 91(1):247-254.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-to birth)-F0- lactation (birth-PND21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674171

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Clinical signs were reported as negative in the text.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. These lack of details are not expected to significantly impact the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. These lack of details are not expected to significantly impact the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CASRN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Animals were pregnant on purchase but parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Pregnant dams were randomly allocated to study groups based on body weight. Male pups were randomly selected from litter for the PUB cohort; the method was not reported.
	Metric 3:	Observational Bias / Blinding Changes	High	Assessors were blinded to treatment groups when assessing AGD and determining areola/nipple numbers and location. The study does not indicate if other endpoints were assessed blindly, although most were non-subjective (body and organ weights) or initial histopathological examination.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with stirring bar removed to avoid contact with plastics.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for most outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block (two blocks included). Results are shown for 13-14 dams/group. The number of male offspring examined is reported in the results tables. There is no indication that any animals were excluded from the analysis.

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest and were justified by the study authors.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. Based on the power analysis conducted by the authors, the number of males analyzed in this cohort was insufficient and lacked the statistical power to adequately detect reproductive histopathological changes. Doses chosen were similar to those previously reported findings in the literature. The methods of endpoint assessments were adequately described, appropriate, and sensitive to the outcomes of interest. Outcomes were assessed consistently across study groups. The test species and strain were appropriate and justified by the study authors.
	Metric 9:	Results presentation	Low	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported and were appropriate in some cases; the litter was used as the statistical unit. Histopathology and gross malformation data for the PUB cohort alone were not adequately reported. Histopathology data were inappropriately combined and analyzed (across study cohorts and blocks, from two exposure durations), which significantly impacts the ability to interpret the study results. Individual animal data were not provided.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observational health of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported for clinical signs.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observational health of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating (from PND 18 to PND 63-65)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Details of outcome assessment were not reported, only that the health of the dams was observed daily. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Clinical signs are reported as negative in text.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (mortality and body weight).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality of pregnant dams-Nutritional/Metabolic-Body weight of pregnant dams, pregnancy weight gain
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations were provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CASRN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Animals were pregnant on purchase but parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Pregnant dams were randomly allocated to study groups based on body weight. Male pups were randomly selected from litter for the PUB cohort; the method was not reported.
	Metric 3:	Observational Bias / Blinding Changes	High	Assessors were blinded to treatment groups when assessing AGD and determining areola/nipple numbers and location. The study does not indicate if other endpoints were assessed blindly, although most were non-subjective (body and organ weights) or initial histopathological examination.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with stirring bar removed to avoid contact with plastics.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for most outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block (two blocks included). Results are shown for 13-14 dams/group. The number of male offspring examined is reported in the results tables. There is no indication that any animals were excluded from the analysis.

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Litter size, pup body weight, and anogenital distance (PND 2); number and location of areola/nipple on PND 13 (all males and females); age and weight of preputial separation (PPS); terminal body weight, body weight on PND 18, body weight gain; serum testosterone and estradiol levels; organ weight (liver, kidney, adrenals, glans penis, ventral prostate, seminal vesicle, levator ani-bulbocavernosus, Cowper's gland, epididymides, testes); whole epididymal sperm count, gross observation for malformations of reproductive organs and histopathology on testes and epididymides.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest and were justified by the study authors.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. Based on the power analysis conducted by the authors, the number of males analyzed in this cohort was insufficient and lacked the statistical power to adequately detect reproductive histopathological changes. Doses chosen were similar to those previously reported findings in the literature. The methods of endpoint assessments were adequately described, appropriate, and sensitive to the outcomes of interest. Outcomes were assessed consistently across study groups. The test species and strain were appropriate and justified by the study authors.
	Metric 9:	Results presentation	Low	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported and were appropriate in some cases; the litter was used as the statistical unit. Histopathology and gross malformation data for the PUB cohort alone were not adequately reported. Histopathology data were inappropriately combined and analyzed (across study cohorts and blocks, from two exposure durations), which significantly impacts the ability to interpret the study results. Individual animal data were not provided.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observational health of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]) with CAS RN 117-81-7 CASRN and lot number. The source and purity (99.1%) of the test substance were reported. Test animal species, strain, sex, age, and source were reported. Parity was not reported. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle) were reported; the number of animals/cage was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were randomly allocated to study groups based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported for clinical signs.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions that were specified seemed to be consistent across groups. Drinking water was filtered (5 m) and tested for Pseudomonas and a suite of chemicals (e.g. pesticides and heavy metals). There was also no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. The study does report that dosing solutions were stored in glass bottles in the dark with the stir bar removed to avoid contact with plastics. This lack of details is not expected to have a significant impact on the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Data were reported for outcomes in tabular form and in the text. The methods report 6-7 pregnant females were dosed per block. Results are shown for 13-14 dams/group.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Gray, L., Barlow, N., Howdeshell, K., Ostby, J., Furr, J., Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. Toxicological Sciences 110(2):411-425.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observational health of dams
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD 8- birth)-F0- lactation (to PND 17)-F1- premating
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697475

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The source and purity (99.1%) of the test substance were reported. Purity was not independently verified. Gavage volume was reported (2.5 ml/kg bw). Dosing solutions were stored in glass bottles in the dark. Preparation of the dosing solutions was not reported. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. Doses chosen were similar to those previously reported findings in the literature. Details of outcome assessment were not reported, only that the health of the dams was observed daily. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Clinical signs are reported as negative in text.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell number, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes being assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were provided for all prespecified outcomes, exposure groups, and time points. No animal attrition was reported (all animals survived the study). Not all animals are accounted for in the results, as most endpoints present a sample size of 4 or 5 or present a range of 4-6 (with a sample size of 6/group reported in the methods). These omissions are not explained but the impact on the results is likely to be minor.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell number, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate considering the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the outcomes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent, and outcome assessment methodologies were described in detail and appropriately addressed the intended outcomes. Testes were fixed in Bouin's solution, which may lead to differential tubular shrinkage. This is not expected to substantially impact results.
	Metric 9:	Results presentation	High	Results are presented for all outcomes using bar graphs that provide adequate detail for interpretation of results, including indicators for statistical significance and error bars. Results are available for all exposure groups and statistical methods were adequately described and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell number, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes being assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were provided for all prespecified outcomes, exposure groups, and time points. No animal attrition was reported (all animals survived the study). Not all animals are accounted for in the results, as most endpoints present a sample size of 4 or 5 or present a range of 4-6 (with a sample size of 6/group reported in the methods). These omissions are not explained but the impact on the results is likely to be minor.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Serum testosterone and luteinizing hormone concentrations, steroidogenic enzyme concentration/activity, Leydig cell number, testes histpathology, Leydig cell stage, mRNA concentrations of Leydig cell specific markers
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate considering the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the outcomes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent, and outcome assessment methodologies were described in detail and appropriately addressed the intended outcomes. Testes were fixed in Bouin's solution, which may lead to differential tubular shrinkage. This is not expected to substantially impact results.
	Metric 9:	Results presentation	High	Results are presented for all outcomes using bar graphs that provide adequate detail for interpretation of results, including indicators for statistical significance and error bars. Results are available for all exposure groups and statistical methods were adequately described and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Death-Nutritional/Metabolic-Body weights and food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes being assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information is not likely to significantly impact these endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for these outcomes (no mortalities and no effects on body weights or food consumption). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Death-Nutritional/Metabolic-Body weights and food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate considering the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the outcomes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodology was not reported, but missing information is not likely to influence the results.
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were observed. Statistical analyses were appropriate, but data were not provided to confirm statistics.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Death-Nutritional/Metabolic-Body weights and food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods for reducing observational bias were not reported. However, the outcomes being assessed are not subjective in nature and therefore a lack of blinding is not expected to substantially impact results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information is not likely to significantly impact these endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for these outcomes (no mortalities and no effects on body weights or food consumption). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Death-Nutritional/Metabolic-Body weights and food consumption
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate considering the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	Dose levels were selected based on results of previous studies that investigated the outcomes of interest. Only two doses were used, which was not sufficient to cover the full range of responses as no NOAEL could be determined. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodology was not reported, but missing information is not likely to influence the results.
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were observed. Statistical analyses were appropriate, but data were not provided to confirm statistics.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Activity of animals
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Low	Methods for reducing observational bias were not reported, and the outcome being assessed is subjective in nature. Therefore, a lack of blinding for this outcome may have impacted study results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for this outcome (no effect on animals' activity). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Activity of animals
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate considering the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	Dose levels were selected based on results of previous studies that investigated the outcomes of interest. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodology was not reported, and the outcome of interest was not described in detail, so it was unclear whether methods were sensitive for the outcome of interest or what the authors intended to evaluate for clinical signs.
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were observed. Statistical analyses were appropriate, but data were not provided to confirm statistics.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Activity of animals
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information was present. The species, dose/concentration levels, duration of exposure, and route were clearly defined. The test article was clearly identified with a chemical name, CASRN, purity and commercial source. Most important information was provided, with the exception of animals' starting body weights and details on animal husbandry conditions. These missing details are not expected to substantially impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The study authors state that animals were randomly allocated into groups, but do not provide details regarding the randomization procedure that was used.
	Metric 3:	Observational Bias / Blinding Changes	Low	Methods for reducing observational bias were not reported, and the outcome being assessed is subjective in nature. Therefore, a lack of blinding for this outcome may have impacted study results.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An adequate vehicle control group was included and responded appropriately. Food consumption and body weights were comparable between control and treated animals. Animal husbandry conditions were not reported, so it cannot be confirmed whether the presence of plasticizers, other phthalates or EDCs in animal housing material were controlled for. The impact of this missing information could be significant for reproductive/developmental endpoints.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Qualitative results were reported for this outcome (no effect on animals' activity). No animal attrition was reported (all animals survived the study) and there is no expected influence on the outcome assessment.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test substance is identified definitively by name, with an appropriate source (Sigma), and with a very high purity of 99%. The authors did not perform an independent analytical verification of test substance purity. Test substance preparation and storage details are not described, but due to the high stability of DEHP, missing information is not likely to have a large impact on the results. Test substance administration is described, and gavage volume is reported (0.5 mL) and is not excessive. Only nominal doses are reported.

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Study Citation:	Guo, J., Li, X. W., Liang, Y., Ge, Y., Chen, X., Lian, Q. Q., Ge, R. S. (2013). The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis. Toxicology 306:9-15.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Activity of animals
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-11-day(s)-Oral-Gavage-Duration: Short-term (>1-30 days)-7-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2001148

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of exposure was sensitive and appropriate considering the purpose of the study to investigate effects of adult exposure on number of Leydig cells in the testis.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	Dose levels were selected based on results of previous studies that investigated the outcomes of interest. Test animals were obtained from a commercial source and the species, strain, and sex were an appropriate model for the outcomes of interest. The number of animals per group was appropriate and consistent. The outcome assessment methodology was not reported, and the outcome of interest was not described in detail, so it was unclear whether methods were sensitive for the outcome of interest or what the authors intended to evaluate for clinical signs.
	Metric 9:	Results presentation	Medium	Only qualitative descriptions were provided for the outcomes, as no effects were observed. Statistical analyses were appropriate, but data were not provided to confirm statistics.

Additional Comments: None

Overall Quality Determination — Medium

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition.Data for body weight and food intake were not appropriately reported. Not all timepoints were reported independently and it cannot be determined which timepoint the data presented pertains to.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Uninformative	Body weight data was reported however it is unclear which time point these data pertain to. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state terminal body weights were recorded for each. Table 1 reports body weight data but does not indicate which timepoint this is for. Lack of this information makes this endpoint uninformative. Mean food intake was reported however which timepoint these values pertain to or how many animals were included in the calculation were not reported. Water intake is reported as an approximation of 7 ml/day.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition.Data for body weight and food intake were not appropriately reported. Not all timepoints were reported independently and it cannot be determined which timepoint the data presented pertains to.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Uninformative	Body weight data was reported however it is unclear which time point these data pertain to. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state terminal body weights were recorded for each. Table 1 reports body weight data but does not indicate which timepoint this is for. Lack of this information makes this endpoint uninformative. Mean food intake was reported however which timepoint these values pertain to or how many animals were included in the calculation were not reported. Water intake is reported as an approximation of 7 ml/day.

Additional Comments: None

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Food consumption, water consumption, and body weight
Duration and Exposure Route:	Oral-Diet-Duration: Short-term (>1-30 days)-7-2-week(s)-Oral-Diet-Duration: Short-term (>1-30 days)-7-4-week(s)-Oral-Diet-Duration: Subchronic (>30-90 days)-7-8-week(s)
Species:	Mouse-A/J - [mouse]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000828

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantitative or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Data for testis weight is not appropriately reported. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state testes weights was recorded for each timepoint. Table 1 reports testes weight data but does not indicate which timepoint this is for. Histopathology of testes was reported sufficiently with means and SD. Statistical analysis was performed and appropriate. Although testicular weight data cannot be used for this assessment, the histopathological data is adequately reported.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantitative or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Data for testis weight is not appropriately reported. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state testes weights was recorded for each timepoint. Table 1 reports testes weight data but does not indicate which timepoint this is for. Histopathology of testes was reported sufficiently with means and SD. Statistical analysis was performed and appropriate. Although testicular weight data cannot be used for this assessment, the histopathological data is adequately reported.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	All details of the test substance (purity, source) were reported. The testing laboratory did not independently verify the purity, but the chemicals sold by Sigma-Aldrich are certified. No information on storage was reported. Both nominal and analytical exposure concentrations were reported. The chamber concentrations were measured daily with a gas chromatograph and the analytical measurement were within 10% of nominal. it was not specified where air was sampled from. Insufficient information was provided regarding the exposure method. The method (whole-body or nose-only) was not specified. Because the methods state that animals were housed 5-6 per cage during treatment and observation periods, it is assumed exposures were whole-body. The authors noted that exposures were carried out in stainless steel gas chambers and that DEHP was "continuously supplied by a special inhalation exposure devise." Because the atmosphere was continuously supplied, it is assumed the chambers were dynamic, but this was not explicitly reported and no further details (e.g., air flow, method of vapor generation) were provided.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were not justified by the study authors, but appeared to be appropriate for the purposes of the study. The study goals were to determine whether exposure at the onset of puberty would have an effect on postbutertal reproductive functions in female rats.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The animal species (female Wister-Imamichi rats) and number of animals per group (n = 10) were appropriate. Two separate experiments were performed, and the endpoints were assessed after two durations of exposure for comparison. The study authors referred to the Female Pubertal Protocol on the influence of prebuteral exposure to endocrine-disrupting chemicals reviewed by Goldman et al (2000): Goldman, J. M., Law, S. C., Balchak, S. K., Cooper, R. L., and Kavlock, R. J. (2000). Endocrine-disrupting chemicals: Prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit. Rev. Toxicol. 30, 135–196 (open access). The authors adequately justified the endpoints assessed and they were sensitive to the outcomes of interest. The dose concentrations/spacing were not clearly justified, but the authors stated that the purpose was to evaluate effects upon exposure to "high air doses." All animals were sampled for the endpoints described.
	Metric 9:	Results presentation	High	Data for all endpoints relevant to these outcomes were presented quantitatively as means ± SE or SD. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means ± SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

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Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means ± SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means ± SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination — Medium

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Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

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

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means ± SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups. There were no differences in food and water intake. The study did not monitor respiratory rates, but the test material is not classified as a respiratory irritant. There were no differences in body weights.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

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

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weights, food and water intake-Hepatic/Liver-Liver weights, serum cholesterol-Renal/Kidney-Kidney weights-Lung/Respiratory-Lung weights-Other (please specify below) (Clinical signs)-Undefined clinical signs
Duration and Exposure Route:	Inhalation-Vapor-Duration: Short-term (>1-30 days)
Species:	Rat-Other (Wistar-Imamichi)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674395

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Body weight data at the time of vaginal opening and first estrous were quantitatively reported as means ± SE. Body weights at other times, food and water consumption and absolute and relative organ weight results were qualitatively reported as negative (no statistical changes) in the text. It was also noted that animals showed no visible signs of toxicity. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	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:	Diethylhexyl 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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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:	Diethylhexyl 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	11784618

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint(s) of interest.
Domain 4: Selective Reporting and Attrition				

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the methods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. Overall, these omissions are not expected to significantly impact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoint of interest. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The sample size (4 animals/group) was small but sufficient to perform statistics. The frequency of body weight measurements was reported and protocols were consistent across groups.
	Metric 9:	Results presentation	Medium	Quantitative data (mean ± SD, represented graphically) were provided for body weights measured at necropsy. Statistical methods were described and were appropriate. Body weight data collected during the exposure period were not reported.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric	Rating	Comments

Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
	Metric 1: Reporting Quality	Low	All critical and some important information was reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance			
	Metric 2: Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3: Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures. Blinding was not reported for clinical signs.
Domain 3: Confounding / Variable Control			
	Metric 4: Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint(s) of interest.
Domain 4: Selective Reporting and Attrition			

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Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the methods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. Overall, these omissions are not expected to significantly impact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoints. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The sample size (4 animals/group) was small and may reduce statistical power. The frequency of animal observations was reported (daily), but no additional methodological details for observing clinical signs were provided (e.g., cage-side; detailed clinical). This is not expected to have a significant impact on the study results.
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for observed clinical signs and abnormal behaviors.

Additional Comments: None

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Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric	Rating	Comments
Overall Quality Determination		**Uninformative**	

Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
	Metric 1: Reporting Quality	Low	All critical and some important information was reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance			
	Metric 2: Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3: Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures. Blinding was not reported for clinical signs.
Domain 3: Confounding / Variable Control			
	Metric 4: Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint(s) of interest.
Domain 4: Selective Reporting and Attrition			

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Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the methods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. Overall, these omissions are not expected to significantly impact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoints. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The sample size (4 animals/group) was small and may reduce statistical power. The frequency of animal observations was reported (daily), but no additional methodological details for observing clinical signs were provided (e.g., cage-side; detailed clinical). This is not expected to have a significant impact on the study results.
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for observed clinical signs and abnormal behaviors.

Additional Comments: None

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Study Citation:	Vo, B., T.T., Jung, E. M., Dang, V. H., Yoo, Y. M., Choi, K. C., Yu, F. H., Jeung, E. B. (2009). Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reproductive Biology and Endocrinology 7:104.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical signs)-Observed clinical signs-Neurological/Behavioral-Abnormal behavior
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-7-15-day(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697420

Domain	Metric	Rating	Comments
Overall Quality Determination		**Uninformative**	

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information was reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Chemical Company); test animal characteristics (species, strain, life stage, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or consisted of an initial histopathology review, and no secondary histopathology review was conducted.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative results were reported for most, but not all outcomes described in the methods. It was stated that "body weights, clinical signs, and abnormal behaviors were recorded daily throughout the experimental period." However, no results were presented for observed clinical signs or abnormal behaviors and only body weights measured at necropsy were provided. The methods state that testes were collected from each group for total RNA isolation; however, results were only reported for the 100 mg/kg-day group. Overall, these omissions are not expected to significantly impact the interpretation of the results. All animals appeared to be accounted for in graphs and there is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Chemical Company), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed. The authors did provide dose amounts in mg/kg bw/day and reported that dosages were adjusted according to changes in body weight. Storage conditions and gavage volume were not reported. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (exposed orally via gavage daily from postnatal day (PND) 21 to 35) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of PND 21-35.
Domain 6: Outcome Measures and Results Display				

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

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an oral toxicity study aimed at examining the effects of DEHP exposure on developing male reproductive organs. The test animals (rats) and sex (males) were appropriate for the evaluation of the endpoints. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate. The number of animals (4 animals/group) was low and likely reduced the statistical power and ability to detect some changes. In addition, for gene expression and histological analyses, it is not clear how many animals were used for each. In the methods section, it is stated that "four testes were collected from each group for total RNA isolation. Other testes were fixed in Bouin's solution, paraffin-embedded, and sectioned at 5 um for histopathological examination." It is not clear if one testis was collected from each experimental animal for RNA isolation and the remaining four testes were used for histological analysis. This uncertainty on the number of animals used to generate the samples is expected to impact the interpretation of gene expression and histological findings. It is unlikely that the sample size was adequate for histopathology.
	Metric 9:	Results presentation	Low	Quantitative data (mean ± SD, represented graphically) were provided for organ weights, anogenital distance, circulating testosterone and LH levels, and some gene expression analysis in the testes. Quantitative data (fold change compared to vehicle) were provided for gene expression results of cDNA microarray analysis for the 100 mg/kg-day group. No microarray results for the low- and high-dose groups were reported. No supplemental files or links to database holdings of the array data were provided. Statistical methods were described and were appropriate for the endpoints. There is a discrepancy regarding the anogenital distance data, the study authors state in the Results and Discussion sections that anogenital distance was significantly reduced in the 500 mg/kg bw/day group as compared to the vehicle. However, this reduction is not apparent on the graph which appears to show an increase in anogenital distance in the low and mid-dose groups, and no change from the negative control in the 500 mg/kg-day group. The statistical analysis of the AGD data is also questionable. The figure suggested statistical analysis may have been mistakenly done using the positive control rather than the negative control. The discrepancies in the anogenital distance results substantially impact the interpretation of that endpoint. Histopathology data were presented as representative figures instead of incidence data.

Additional Comments: None

Overall Quality Determination Low

Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells
Duration and Exposure Route:	Oral-Gavage-Duration: Subchronic (>30-90 days)-7-70-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study does 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 or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for most prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells
Duration and Exposure Route:	Oral-Gavage-Duration: Subchronic (>30-90 days)-7-70-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Results for most endpoints were described in the text and data were presented in tables as means ± standard error. Statistical tests were reported and appropriate. Histopathological data were reported as negative in the text.

Additional Comments: Study included 3 different durations of exposure during post weaning up to adulthood. each ”experiment” included different outcomes and were potentially performed on different cohorts of animals (though this is unclear and all groups were >10). not all outcomes were evaluated for each duration. This evaluation corresponds with the PND21-90 duration

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition.Data for body weight and food intake were not appropriately reported. Not all timepoints were reported independently and it cannot be determined which timepoint the data presented pertains to.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Uninformative	Body weight data was reported however it is unclear which time point these data pertain to. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state terminal body weights were recorded for each. Table 1 reports body weight data but does not indicate which timepoint this is for. Lack of this information makes this endpoint uninformative. Mean food intake was reported however which timepoint these values pertain to or how many animals were included in the calculation were not reported. Water intake is reported as an approximation of 7 ml/day.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantitative or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Data for testis weight is not appropriately reported. Three exposure durations were studied (2, 4, and 8 weeks) and the methods state testes weights was recorded for each timepoint. Table 1 reports testes weight data but does not indicate which timepoint this is for. Histopathology of testes was reported sufficiently with means and SD. Statistical analysis was performed and appropriate. Although testicular weight data cannot be used for this assessment, the histopathological data is adequately reported.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	All animals were accounted for in Table 2. There is no indication that any animals died or were not included in analysis. There is no indication of animal attrition. Quantitative or qualitative results were reported for most, but not all outcomes described in the methods. Data for testes weight was not appropriately reported. It cannot be determined which timepoint the data pertains to. All other endpoints described in the methods were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity of the test substance was reported and DEHP-dosed chows were purchased from a company. It is unclear whether the laboratory performing the study independently analytically verified the test article purity and composition. In addition, the test substance concentrations in the chow were not analytically confirmed. Storage conditions and stability of the DEHP-dosed chow were not reported. The route and method of exposure were suited to the test substance. The authors report the calculated dose/day as a range. It is unclear if these dose ranges are based on food intake and body weight measurements from animals used in this study. The lack of detail on test substance characterization and uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	This was an oral toxicity study. The test animals (mice) and sex (males) were appropriate for evaluation of the endpoints. Although the number of exposure groups (0, 0.01%, 0.1% DEHP) was lower than is recommended for the study type (OECD Guideline 407), the study authors justified their dose selection and concentration spacing based on existing toxicity data. The sample size (10 animals/group/duration) was appropriate for the study type. Outcome assessment methodology was appropriate and assessed consistently across study groups.
	Metric 9:	Results presentation	High	Lymphocyte infiltration was reported as means with SD. Immunohistochemistry and mRNA expression were fully reported. Statistical analysis was performed and appropriate.

Additional Comments: None

Overall Quality Determination Medium

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

Domain	Metric	Rating	Comments

Study Citation:	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19)
Duration and Exposure Route:	Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s)
Species:	Rat-Wistar - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674255

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 di (2-ethylhexyl) phthalate (DEHP). The source and purity (99.9% pure) were reported. Test animal species, strain, sex, age and source were reported. Initial body weight of the animals was not reported. Rats were housed under controlled temperature (exact temperature and humidity not reported) and lighting conditions with a 12 hr day: night cycle. Food and water were available ad libitum. Number of animals/cage were not reported. The concentration levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with qualitative and quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for Metric 2. Allocation of animals to dose groups was done by body weight randomization to ensure equal weight distribution among the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	This study is considered High for Metric 2.2. Histological examination of testis was performed blinded to the treatment group. Other endpoint evaluated were not blinded, but were not subjective in nature (body weight, serum levels of hormones, organ weights, level of mRNA).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	This study is considered Low for Metric 3. The negative control group was appropriate (exposed to air under identical conditions). There is no indication of infection, or any other health condition occurred in the animals. The study does not report the type of cage or water bottle the animals were provided. Co-exposure to plasticizers should be avoided when studying endocrine disruptors such as DEHP because they have the potential to confound the effects of the chemical of interest. It is not clear if steps were made to avoid exposure to plastics, therefore this metric is rated as low.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	This study is considered High for Metric 4. The study does not indicate any animals died. All animals that were exposed were accounted for in the results. Data were provided for all outcomes of interest discussed in the methods.

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Study Citation:	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19)
Duration and Exposure Route:	Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s)
Species:	Rat-Wistar - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674255

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	This study is considered High for Metric 5.1. The purity of the test substance is reported to be 99.9%. A dynamic chamber was used with 15 air changes/hour. DHEP vapor concentration was measured once a day via gas chromatography. The mean concentration with variance were reported as 5.1+/- 1.3 and 24.6 +/-5.2 mg/m3. Given the variance of >10% of the mean, there is some uncertainty with the exposure. Also, the study does not report at what point in the exposure measurements were made. Only recording one measurement over the six hours invites some uncertainty as to the consistency of exposure throughout the exposure time.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5.2. In this inhalation study, the route, frequency, and duration of exposure (6 hours/day, 5 days/week, for 4 or 8 weeks) were appropriate for the study type and outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered Medium for Metric 6.1. Two exposure groups were studied at two different timepoints. A full range of responses were not obtained; a LOAEL was obtained, but a NOAEL was not. The study did not explain the reasoning for choosing these concentrations. The outcome assessment methodology was adequately reported and cited in HERO 2850042; Lanning et al. 2002. Outcomes were assessed consistently across study groups and appropriate for study interest.
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Body weight, organ weight, serum hormone levels and mRNA expression are all fully reported with mean, variance and n at both timepoints. Histological findings are reported in text (without incidence data). The proportion of immature tubules is fully reported using grading system. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19)
Duration and Exposure Route:	Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s)
Species:	Rat-Wistar - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674255

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 di (2-ethylhexyl) phthalate (DEHP). The source and purity (99.9% pure) were reported. Test animal species, strain, sex, age and source were reported. Initial body weight of the animals was not reported. Rats were housed under controlled temperature (exact temperature and humidity not reported) and lighting conditions with a 12 hr day: night cycle. Food and water were available ad libitum. Number of animals/cage were not reported. The concentration levels, frequency, duration, and route of exposure were reported. Endpoint evaluation methods were reported along with qualitative and quantitative data. All critical information is provided and although some important information is missing, the missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	This study is considered High for Metric 2. Allocation of animals to dose groups was done by body weight randomization to ensure equal weight distribution among the groups.
	Metric 3:	Observational Bias / Blinding Changes	High	This study is considered High for Metric 2.2. Histological examination of testis was performed blinded to the treatment group. Other endpoint evaluated were not blinded, but were not subjective in nature (body weight, serum levels of hormones, organ weights, level of mRNA).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	This study is considered Low for Metric 3. The negative control group was appropriate (exposed to air under identical conditions). There is no indication of infection, or any other health condition occurred in the animals. The study does not report the type of cage or water bottle the animals were provided. Co-exposure to plasticizers should be avoided when studying endocrine disruptors such as DEHP because they have the potential to confound the effects of the chemical of interest. It is not clear if steps were made to avoid exposure to plastics, therefore this metric is rated as low.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	This study is considered High for Metric 4. The study does not indicate any animals died. All animals that were exposed were accounted for in the results. Data were provided for all outcomes of interest discussed in the methods.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Kurahashi, N., Kondo, T., Omura, M., Umemura, T., Ma, M., Kishi, R. (2005). The effects of subacute inhalation of di (2-ethylhexyl) phthalate (DEHP) on the testes of prepubertal Wistar rats. Journal of Occupational Health 47(5):437-444.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight-Reproductive/Developmental-Serum testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH), organ weights (testes, epididymis, seminal vesicles and ventral prostate), histology on testis (histopathologic changes and progression of spermatogenesis), and testicular mRNA levels of enzymes involved in testosterone biosynthesis (P450scc, 3B-HSD, CYP17 and CYP19)
Duration and Exposure Route:	Inhalation-Vapor-Duration: Subchronic (>30-90 days)-5-6-8-week(s)
Species:	Rat-Wistar - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674255

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	This study is considered High for Metric 5.1. The purity of the test substance is reported to be 99.9%. A dynamic chamber was used with 15 air changes/hour. DHEP vapor concentration was measured once a day via gas chromatography. The mean concentration with variance were reported as 5.1+/- 1.3 and 24.6 +/-5.2 mg/m3. Given the variance of >10% of the mean, there is some uncertainty with the exposure. Also, the study does not report at what point in the exposure measurements were made. Only recording one measurement over the six hours invites some uncertainty as to the consistency of exposure throughout the exposure time.
	Metric 7:	Exposure timing, frequency, and duration	High	This study is considered High for Metric 5.2. In this inhalation study, the route, frequency, and duration of exposure (6 hours/day, 5 days/week, for 4 or 8 weeks) were appropriate for the study type and outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This study is considered Medium for Metric 6.1. Two exposure groups were studied at two different timepoints. A full range of responses were not obtained; a LOAEL was obtained, but a NOAEL was not. The study did not explain the reasoning for choosing these concentrations. The outcome assessment methodology was adequately reported and cited in HERO 2850042; Lanning et al. 2002. Outcomes were assessed consistently across study groups and appropriate for study interest.
	Metric 9:	Results presentation	Medium	This study is considered High for Metric 6.2. Body weight, organ weight, serum hormone levels and mRNA expression are all fully reported with mean, variance and n at both timepoints. Histological findings are reported in text (without incidence data). The proportion of immature tubules is fully reported using grading system. Statistical analysis was appropriate.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

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

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Data for all endpoints relevant to these outcomes were presented quantitatively as means ± SE or SD. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

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

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Data for all endpoints relevant to these outcomes were presented quantitatively as means ± SE or SD. The methods of statistical analysis were clearly reported and adequate.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other outcomes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

Domain 6: Outcome Measures and Results Display

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

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

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other outcomes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

Domain 6: Outcome Measures and Results Display

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

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

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other outcomes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

Domain 6: Outcome Measures and Results Display

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

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

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	The study reported all critical and important information. The test material was DEHP (purity 99%). The CASRN and the commercial source were reported. Animal species, strain, age, source, and body weights were reported. Animal husbandry conditions (temperature, humidity, lighting), food and water availability, and the number of animals per cage were reported. Animals were exposed via inhalation and the number of animals per group, exposure durations, and concentrations were clearly reported. Endpoint evaluation methods were clearly described, and quantitative or qualitative results were reported for all outcomes specified in the methods.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were ranked by body weight for placement into treatment groups such that the mean body weights were similar across groups. No random allocation methods were described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, but risk of bias is mitigated because the endpoints were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group (0 mg/m3) was included, but no details on the generation of the exposure atmospheres were provided, and it was not explicitly stated that the control animals were exposed to air only, or that they were concurrent. Animal husbandry conditions appeared to be consistent across groups; however, food and water intake were not monitored for this experiment. Significant reductions in body weights were observed, but based on the discussion section, a similar reduction has been reported in other studies and was considered to be a treatment-related effect.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Results for these outcomes (organ weights), results were qualitatively reported as negative, and therefore sample size cannot be determined. However, for other outcomes/endpoints in this study, all animals were accounted for in the results. There were no mortalities and no evidence of selective reporting or attrition.
Domain 5: Exposure Methods Sensitivity				

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

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

Domain 6: Outcome Measures and Results Display

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

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

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells
Duration and Exposure Route:	Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study does 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 or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for most prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular weight, serum estradiol, testosterone, and luteinizing hormone levels; ex vivo production of testosterone and estradiol from isolated Leydig cells (basal and after LH stimulation); Leydig cell proliferation (assessed by 1) mRNA expression of cell division cycle markers (PCNA, cyclin D3 and G1, and tumor suppressor protein p53); 2) tritiated thymidine incorporation in purified Leydig cells; 3) counting the number of Leydig cells in testis by stereology); aromatase gene expression in Leydig cells
Duration and Exposure Route:	Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	Medium	Results for most endpoints were described in the text and data were presented in tables as means ± standard error. Statistical tests were reported and appropriate. Histopathological data were reported as negative in the text.

Additional Comments: Study included 3 different durations of exposure during post weaning up to adulthood. each ”experiment” included different outcomes and were potentially performed on different cohorts of animals (though this is unclear and all groups were >10). not all outcomes were evaluated for each duration. This evaluation corresponds with the PND21-120 duration

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (di(2-ethylhexyl)phthalate [DEHP]), CAS RN was not provided. The source and purity of the test substance were not reported. Test animal species, strain, sex, and age were reported. Source of the animals was not provided. Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were not reported. Food and water availability were not reported. Route of exposure, duration and doses were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study does 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 or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature (e.g. body weight, serum levels, cell counts).
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included and responses were appropriate. A positive control group is not necessary for this type of study. Housing conditions were not reported. There was also no indication of whether test animal bedding or food were analyzed for the presence of contaminants, such as phthalates. Food and water dispensing containers were not described. However, the missing information is not expected to significantly impact the endpoints described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative data were presented for all prespecified outcomes. The study methods report at least 10 animals/group were exposed. Data for some endpoints are reported as 10 animals/group (other endpoints do not provide the n for the data); it is unclear if some animals may have been excluded.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance were not reported. Gavage volume was not reported. No information is provided on preparation or storage of the test substance. It is unclear how far in advance solutions were made. Only target concentrations are provided, and doses were not analytically verified.

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Study Citation:	Akingbemi, B. T., Ge, R., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America 101(3):775-780.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight
Duration and Exposure Route:	Oral-Gavage-Duration: Chronic (>90 days)-7-100-day(s)
Species:	Rat-Long-Evans - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673552

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The timing and duration of exposure were appropriate for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough (comparable to recommended by guidance, 10) and sufficient for statistical analysis. The doses were chosen based on reported findings by this study group. Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups.
	Metric 9:	Results presentation	High	Results were reported as means ± standard error. Statistical tests were reported and appropriate.

Additional Comments: Study included 3 different durations of exposure during post weaning up to adulthood. each ”experiment” included different outcomes and were potentially performed on different cohorts of animals (though this is unclear and all groups were >10). not all outcomes were evaluated for each duration. This evaluation corresponds with the PND21-120 duration

Overall Quality Determination — Medium

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body Weight
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body Weight
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. The outcome assessment methodology (measuring body weight throughout the study) addressed the outcome of interest and from the information we have, it appears that it was applied consistently. The sampling was adequate for the intended outcome.
	Metric 9:	Results presentation	Low	Body weight data was presented in a figure presumably showing means with no measures of variance. Statistical analysis was performed but statistical methods were not adequately described. Individual animal data were not provided.

Additional Comments: None

Overall Quality Determination — Low

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testes histology and cell appearance; testes function as measured by inhibition of spermatogenesis and instances of general tubular atrophy
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of the test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. Insufficient information has been provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard. The study did not specify whether measures were taken to reduce exposure to other plasticizers, and this may have a significant impact on endpoints affected by endocrine disruption.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testes histology and cell appearance; testes function as measured by inhibition of spermatogenesis and instances of general tubular atrophy
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. The outcome assessment methods for conducting histopathology were not reported. Other endpoints to assess the toxicity of the target organ (e.g., organ weights) were not evaluated. The sample sizes were not specified for histopathology and the number of animals per group is unclear. Animal source and age were not reported.
	Metric 9:	Results presentation	Uninformative	Exposure-related effects were qualitatively described in the text (testis histology). Statistical methods were not described, and the statistical significance of the changes is unknown. No data are available for an independent analysis.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Cancer/Carcinogenesis-Tumors-Other (please specify below) ("general health")-Assessment of "general health"
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Cancer/Carcinogenesis-Tumors-Other (please specify below) (”general health”)-Assessment of ”general health”
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. The outcome assessment methodologies for clinical observations were missing and details of histological examinations were insufficient. Sample sizes were not specified for either outcome and the number of animals per group is unclear. Animal source and age were not reported.
	Metric 9:	Results presentation	Medium	Animals were qualitatively reported to be ”in good health” throughout the study. The text was suggestive that no treatment-related tumors were observed, but no data were provided.

Additional Comments: None

Overall Quality Determination — Low

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Cancer/Carcinogenesis-Tumors-Other (please specify below) ("general health")-Assessment of "general health"
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed diets in the absence of test substance. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Cancer/Carcinogenesis-Tumors-Other (please specify below) (”general health”)-Assessment of ”general health”
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. The outcome assessment methodologies for clinical observations were missing and details of histological examinations were insufficient. Sample sizes were not specified for either outcome and the number of animals per group is unclear. Animal source and age were not reported.
	Metric 9:	Results presentation	Medium	Animals were qualitatively reported to be ”in good health” throughout the study. The text was suggestive that no treatment-related tumors were observed, but no data were provided.

Additional Comments: None

Overall Quality Determination — Low

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl-transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) is reported. Important information reported included the test substance source and purity, test animal life stage (adult), starting body weight, and method of exposure. Animal source and exact age at the start of the study was not reported. The study specified that care was taken to regulate "environmental factors including room temperature and humidity, cage contents, and cleanliness," but no specific animal husbandry details were reported, including the number of animals per cage. The number of animals per group is not clearly stated, although sample sizes were generally specified. Limited endpoint evaluation methods were provided. There is a significant amount of missing information or lack of reported details that is expected to have a significant impact on the study results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measure to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a concurrent negative control group fed normal diets. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study and there were dose-related decreases in animal body weights. There is insufficient information provided to determine whether palatability was a contributing factor. Animal husbandry details were not explicitly reported, but the study authors did describe efforts to keep the conditions standard.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The study does not report mortality or survival rates in a chronic 102-week-long study. The text specified that "the general health of all rats in both the control and treated groups appeared to be good throughout the entire investigation period." The authors do not specifically report animal attrition or omissions, other than stating that only 2 of the 520 rats used in the entire study developed spontaneous mammary cancer. Results from this endpoint are presented for each exposure group at each timepoint.

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl-transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-24-102-week(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Feed intake was not measured; limited measurements of mean body weights throughout the study were reported in a figure and could be used to estimate doses in mg/kg-day if using default food consumption values.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, duration and frequency are clearly reported and appropriate for the study given the goals of the authors (assessing the effects of prolonged exposure).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The number of exposure groups and dose space were appropriate for the purpose of the study (effect of prolonged, low-level exposure to test article on hepatic enzyme function). The authors clearly justified the use of low exposure levels. Animal source and age were not reported. Methodological details for conducting electron microscopy were adequately described. Methods for isolation of hepatic fractions and enzyme measurements were cited to other studies that were not available for review. Protein determination was done according to Lowry et al. (1951), which is a known and accepted method. Sample sizes ranged from 9-14 or from 6-11 for most endpoints. No details on sampling were provided. Histopathology details were insufficiently described.
	Metric 9:	Results presentation	Low	Results for liver-related endpoints were not adequately reported. Representative images of electron microscopy findings were provided along with detailed descriptions in the text. These data were not statistically analyzed. Protein and enzyme activity data was presented in figures presumably reporting mean values with no measures of variance. Additional "statistical evaluation" tables were included to specify statistical significance, but the statistical methods used were not described, and individual animal data were not available to conduct an independent analysis. The lack of histopathology findings was stated in the study text.

Additional Comments: None

Overall Quality Determination — Low

Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl-transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-1-year(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	Critical information (SD rats, test article identified by name, dose level, duration of exposure, exposure route, and qualitative or quantitative results for at least one endpoint) was reported; however, no additional details were provided. Neither the number of animals per group or the sample size was specified.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated into groups or how animals were selected for the various times of sacrifice.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Measures to reduce observational bias are not described but the potential concern was mitigated because the outcomes were not subjective and based on simple objective measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group fed diets in the absence of test substance; however, it does not appear that control animals were included during the post-exposure period. Positive controls are not required for the study type. Food consumption was not measured directly in a dietary study.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	Insufficient information was provided to determined attrition or selective reporting.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Uninformative	The test material source (Fluke AG) and purity (>99%) were reported. No certificate of analysis was included but likely was available from the supplier at the time of purchase. The test substance was not analytically verified by the performing laboratory. Animals were exposed via the diet. No details on the preparation of the diets were provided including no details on the frequency of preparation, homogeneity, or storage. There is significant uncertainty in the dosing. The study reported % DEHP in the diets; the concentrations were not analytically verified. Neither feed intake nor body weights were measured and reliable doses in mg/kg-day cannot be determined.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure timing and frequency were reported. Animals were exposed via the diet for 1 year. No justification was provided by the study author.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Ganning, A. E., Olsson, M. J., Brunk, U., Dallner, G. (1990). Effects of prolonged treatment with phthalate ester on rat liver. Pharmacology & Toxicology 67(5):392-401.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver histology, (both light and electron microscopy); liver enzyme activities: catalase and palmitoyl-CoA (homogenate), carnitine acetyl-transferase and cytochrome oxidase (mitochondria), and CYP-450, NADH and NADPH cytochrome c reductase (microsomes)
Duration and Exposure Route:	Oral-Diet-Duration: Chronic (>90 days)-7-1-year(s)
Species:	Rat-Sprague-Dawley - [rat]-Male
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	679540

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Uninformative	The study included a single endpoint (measurement of hepatic enzyme activities). It is unclear what the purpose of the study was. Enzyme activities alone are not considered to be a sensitive endpoint for assessing hepatic toxicity. No methodological details were provided, although similar measurements were reported for another experiment reported in the same study. Methods of enzyme measurements were cited to other sources.
	Metric 9:	Results presentation	Uninformative	Data were reported in figures showing means without measures of variance for the control and high-dose groups only. Results for the 0.2% group were briefly described in the study text. It does not appear that statistical analysis was conducted and insufficient information was provided to conduct an independent analysis.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Laws, M. J., Meling, D. D., Deviney, K., A.R., Santacruz-Márquez, R., 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	11784618

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”. Male offspring were ”randomly obtained from seven dams in each group at each stage,” but the method of randomization was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food intake, body weights, organ weights, hormone concentrations) or did not require blinding (histology)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during GD 12-21 which is the relevant period for male sexual differentiation (Ema et al. 1993). Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 414 Guideline which focuses on developmental toxicity recommends using more than 16 animals. In this study, there was a total of 7 dams and 9-18 offspring. The study authors did not justify the number of animals per group and sample sizes; however, sample sizes were sufficient to allow for statistical analysis. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Outcome methodologies for the offspring were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Low	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs, however other endpoints (e.g., organ weights) were only reported as negative in the text and data were not shown. Statistical analysis methods were reported and statistical significance was noted in tables. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided. As noted by Dishaw et al., 2020, the presentation of offspring data as means of individual animals, rather than as litter means, has the potential to overestimate the statistical significance of experimental findings.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0- lactation (PND 1-21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”. Male offspring were ”randomly obtained from seven dams in each group at each stage,” but the method of randomization was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food intake, body weights, organ weights, hormone concentrations) or did not require blinding (histology)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Leydig cell testosterone production, serum testosterone and LH measurements, testicular histology and weight, seminal vesicles weight
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0- lactation (PND 1-21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during the lactation period, which was appropriate for the outcomes of interest. Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The OECD 414 Guideline which focuses on developmental toxicity recommends using more than 16 animals. In this study, there was a total of 7 dams and 9-18 offspring. The study authors did not justify the number of animals per group and sample sizes; however, sample sizes were sufficient to allow for statistical analysis. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Outcome methodologies for the offspring were adequately reported and sensitive for the endpoints assessed. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted.
	Metric 9:	Results presentation	Low	Results for developmental endpoints were shown in tables (shown as means ± SEM) or graphs, however other endpoints (e.g., organ weights) were only reported as negative in the text and data were not shown. Results for body weight were presented in a table (shown as means ± SEM). Food consumption results was not reported for this experiment. Statistical analysis methods were reported and statistical significance was noted in tables. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided. As noted by Dishaw et al., 2020, the presentation of offspring data as means of individual animals, rather than as litter means, has the potential to overestimate the statistical significance of experimental findings.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”. Male offspring were ”randomly obtained from seven dams in each group at each stage,” but the method of randomization was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food intake, body weights, organ weights, hormone concentrations) or did not require blinding (histology)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates; This is not expected to have a significant impact on the endpoint of interest (e.g., body weights). Cages, food containers, and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)-F0- lactation
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during GD 12-21 which is the relevant period for male sexual differentiation (Ema et al. 1993). Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The number of animals per group and sample size were appropriate for the outcome of interest. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted. The methods did not specify the timing of body weight and feed intake measurements, but the results noted body weights were measured at the beginning and end of the exposure.
	Metric 9:	Results presentation	Medium	Dam body weight measurements were quantitatively reported as means and unspecified measures of variance. Food intake results were not reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0- lactation (PND 1-21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name di(2-ethylhexyl)phthalate (DEHP). A CASRN was not provided. The source and purity (>99%) were reported. Test animal species, strain, sex, and source were reported. Age was not specified upon receipt of animals. Rats were timed-pregnant from the supplier. Initial body weights were not reported. Husbandry conditions (light cycle, number of animals/cage) were reported. Cage type, bedding type, temperature, and humidity were not reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as mg/kg/day. The number of dams and offspring/group were reported. The frequency was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were allocated by body weight randomization to “ensure equal weight distribution between groups”. Male offspring were ”randomly obtained from seven dams in each group at each stage,” but the method of randomization was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints were not subjective in nature (food intake, body weights, organ weights, hormone concentrations) or did not require blinding (histology)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative control group was included (vehicle only) and responses were appropriate. Housing and treatment conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates. Cages, food containers, and water dispensing containers were not described. This is not expected to have a significant impact on the endpoint of interest (body weight).
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in text or table. Food intake results were not reported. Table footnotes specified the number of animals included in each group for analysis; however, complete litter data was not reported. Therefore, it is unclear if all animals were evaluated and included in the analysis and the numbers were not consistent across groups evaluation time points.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Akingbemi, B. T., Youker, R. T., Sottas, C. M., Ge, R., Katz, E., Klinefelter, G. R., Zirkin, B. R., Hardy, M. P. (2001). Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biology of Reproduction 65(4):1252-1259.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food consumption in dams and young adult rats
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0- lactation (PND 1-21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673553

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Certificates of analysis are available from the supplier upon request; the test substance was not analytically verified by the performing laboratory. No details were provided on preparation or storage of the test material. Target test concentrations were reported; there is no indication that analytical confirmation was done. Gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure was during GD 12-21 which is the relevant period for male sexual differentiation (Ema et al. 1993). Exposure was consistent across study groups. Groups were treated concurrently.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The test animal was appropriate for the evaluation of the outcomes of interest. The number of animals per group and sample size were suitable for the outcome of interest. Only a single dose group was tested, but the purpose of the study was to assess different sacrifice times after exposure. Dose rationale was not specified although previous studies using DEHP and a developmental study using another phthalate were consulted. The methods did not specify the timing of body weight and feed intake measurements, but the results noted body weights were measured at the beginning and end of the exposure.
	Metric 9:	Results presentation	Medium	Dam body weight measurements were quantitatively reported as means and unspecified measures of variance. Food intake results were not reported.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
	Metric 1: Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq$ 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 $\pm$ 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.
Domain 2: Selection and Performance			
	Metric 2: Allocation	Low	It was not specified how dams were allocated into dose groups, and there is no indication that methods were taken to minimize selection bias (e.g., normalization for body weights). However, a referenced study by the same group HERO 674171 did specify that females were randomly assigned, but the method was not reported. It is not clear though, that the same was done for this study. Randomization was included for specific endpoints. For testicular morphometry, ten randomly chosen fields were analyzed for each animal, and 20 round seminiferous tubule cross-sections were randomly chosen for each animal to count Sertoli cell nucleoli. The study included a male offspring mating experiment. It was not indicated whether the male offspring were randomly selected for mating on PND 110, or for sexual behavioral assessments.

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

Domain	Metric		Rating	Comments
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded; the measurements for most of the endpoints in this outcome were not subjective in nature (e.g., measurements of serum testosterone, organ weights, or fell under initial histopathology examinations (morphometry examinations); however, at least one of the endpoints in this outcome could be subjective in nature (sertoli cell counts). The methods used for the cell counts (e.g., hemocytometer) were not specified.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortality, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers reflected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes specified in the methods were reported in a quantitative or qualitative manner.
Domain 5: Exposure Methods Sensitivity				

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

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

Domain 6: Outcome Measures and Results Display

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

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The endpoints were sensitive and specific for the outcomes of interest and were consistent with the purpose of the study which was to "investigate possible long-term effects of developmental exposure on the male reproductive tract structure and function." The methods are generally adequately described; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 ± 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't specified which groups were sacrificed when. After sacrifice, analysis of serum testosterone, subsequence gross examinations, organ weights, body weight, histopathology, testis morphometry, sperm cell counts etc. were conducted. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a significant impact on all outcomes and makes it difficult to interpret the results shown. Additionally, it is not clear whether the animals sacrificed at this time were unmated or previously mated, and it is unknown if mating status was consistent across groups. – For example, a subset of 110-day-old male offspring (N = 16-18 per group) were mated/paired to unexposed females for 14 days. On PND 130 "experienced" males (n = 14-17 per group were also mated to unexposed females to assess sexual behavior. On PND 144 ± 7 days, adult male offspring (n = 19-20/dose) were sacrificed to assess the other reproductive endpoints described above. It was not specified whether these males sacrificed at ~ PND 144 were unmated, or if this group included some mated males used previously. This is a significant oversight in detail because whether or not the males had been mated, and how soon they were sacrificed post-mating, could significantly impact the results. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple outcomes.

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

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	The adequacy of the data reporting of reproductive effects varies by endpoint. Reproductive organ weights, and serum testosterone levels were reported quantitatively as means ± SE. Statistical methods were adequately described. Likewise, daily sperm production and sperm and testicular morphometry, and testicular cell count data were quantitatively reported in an acceptable manner. In all cases, outliers (macroscopically small testes/epididymides, and one extremely enlarged testis) were excluded from the analysis. Reproductive performance data, fetal data (e.g., viable fetuses, fetal body weights), and sexual behavior data were all quantitatively reported with adequate descriptions of statistical analysis. Results for some reproductive endpoints were described qualitatively (reproductive tract malformation) or semi-quantitatively (testis-histopathology) in the text with a representative figure showing the observed effects for the latter. Incidences were not reported for every observed effect, although dose groups were specified. It does not appear that data for these endpoints were statistically analyzed and are not reported in a manner that would allow for an independent analysis.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-97.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative statement of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673565

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq$ 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 $\pm$ 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indication that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was mitigated for these outcomes (organ weights) because the measurements were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-97.
Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative statement of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673565

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortality, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers reflected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes specified in the methods were reported in a quantitative or qualitative manner.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.
Domain 6: Outcome Measures and Results Display				

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Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative statement of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (From GD 6)-F0- lactation (through LD 21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673565

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	Organ weights (liver, kidney, spleen, and thymus) alone, in the absence of histopathology, is not the most sensitive endpoint for identifying organ-specific toxicity; however, the purpose of the study was to evaluate reproductive effects and recording the weights of these other organs seemed to be an aside. The methods are generally adequately described; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 $\pm$ 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't specified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a significant impact on the organ weight results and makes it difficult to interpret the results reported. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple outcomes.
	Metric 9:	Results presentation	Medium	Negative effects on liver, kidney, spleen, and thymus organ weights were qualitatively reported in the text. Based on the methods, statistical analysis for liver and kidney weights was analyzed with body weight as a co-variate, but the spleen and thymus were evaluated without adjustment due to an absence of meaningful correlation between those organs and body weight. Magnitudes of changes were not reported, so it cannot be determined whether there were any biologically relevant changes.

Additional Comments: None

Overall Quality Determination — Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq$ 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 $\pm$ 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indication that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was mitigated for these outcomes (organ weights) because the measurements were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.
Domain 4: Selective Reporting and Attrition				

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Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
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Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortality, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers reflected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes specified in the methods were reported in a quantitative or qualitative manner.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.
Domain 6: Outcome Measures and Results Display				

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Health Outcome(s) and Reported Health Effect(s):	Hepatic/Liver-Liver weights (data not shown; qualitative statement of negative findings)-Renal/Kidney-Kidney weights (data not shown; qualitative statement of negative findings)-Immune/Hematological-Spleen and thymus weights (data not shown; qualitative statement of negative findings)
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Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673565

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	Organ weights (liver, kidney, spleen, and thymus) alone, in the absence of histopathology, is not the most sensitive endpoint for identifying organ-specific toxicity; however, the purpose of the study was to evaluate reproductive effects and recording the weights of these other organs seemed to be an aside. The methods are generally adequately described; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 $\pm$ 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't specified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a significant impact on the organ weight results and makes it difficult to interpret the results reported. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple outcomes.
	Metric 9:	Results presentation	Medium	Negative effects on liver, kidney, spleen, and thymus organ weights were qualitatively reported in the text. Based on the methods, statistical analysis for liver and kidney weights was analyzed with body weight as a co-variate, but the spleen and thymus were evaluated without adjustment due to an absence of meaningful correlation between those organs and body weight. Magnitudes of changes were not reported, so it cannot be determined whether there were any biologically relevant changes.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228(1):85-97.
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Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673565

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq$ 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 $\pm$ 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indication that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was mitigated for these outcomes (organ weights) because the measurements were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.
Domain 4: Selective Reporting and Attrition				

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Species:	Rat-Wistar - [rat]-Female
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Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	The study did not provide enough information to determine whether attrition occurred. This study did not include any observations of treated dams (e.g., clinical signs, mortality, etc) as part of the study. The number of treated dams was not clearly specified but generated 11 to 16 litters per group. Because observations in dams, including mortality were not included in the study, it is not known whether the differing litter numbers reflected any possible attrition in the dams. For later experiments using male offspring, the total number of male offspring per litter was not specified, only the numbers of offspring sampled for different outcomes. There is insufficient information to determine whether any animal attrition or selective reporting occurred. Results for all of the outcomes specified in the methods were reported in a quantitative or qualitative manner.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurements were conducted (e.g., once, for every dose, etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing) or storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population. The highest dose was known to induce adverse reproductive effects in male offspring without causing overt maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on the frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.
Domain 6: Outcome Measures and Results Display				

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Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
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Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	Organ weights (liver, kidney, spleen, and thymus) alone, in the absence of histopathology, is not the most sensitive endpoint for identifying organ-specific toxicity; however, the purpose of the study was to evaluate reproductive effects and recording the weights of these other organs seemed to be an aside. The methods are generally adequately described; however, there were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 $\pm$ 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't specified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a significant impact on the organ weight results and makes it difficult to interpret the results reported. Although the number of animals used for each outcome was appropriate for statistical analysis and was representative of all litters, no explanations were provided regarding the ranges. Different numbers/ranges of animals were used for multiple outcomes.
	Metric 9:	Results presentation	Medium	Negative effects on liver, kidney, spleen, and thymus organ weights were qualitatively reported in the text. Based on the methods, statistical analysis for liver and kidney weights was analyzed with body weight as a co-variate, but the spleen and thymus were evaluated without adjustment due to an absence of meaningful correlation between those organs and body weight. Magnitudes of changes were not reported, so it cannot be determined whether there were any biologically relevant changes.

Additional Comments: None

Overall Quality Determination — Medium

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Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Female Wistar rats (number not clearly specified) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through lactation day 21 (LD21), generating 11-16 litters per group. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq$ 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 $\pm$ 15g). The parity and age of the animals were not specified. No animal husbandry details, including the number of animals per cage, or how litters were housed as offspring were raised to adulthood, were provided. However, the reader is referred to another study by the same Authors for animal husbandry details (HERO 674171). This reference reports room conditions (temperature, humidity, lighting), food and water availability, cage types, and how animals were housed. Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	It was not specified how animals were allocated into dose groups, and there is no indication that methods were taken to minimize selection bias (e.g., normalization for body weights). A referenced study by the same group: HERO 674171 did report randomizing animals into groups, but the method of randomization was not specified, and it is unclear whether the same was done for this study.
	Metric 3:	Observational Bias / Blinding Changes	Medium	It was not specified whether assessors were blinded, but the potential for bias was mitigated for this outcome (body weights) because the measurements were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. For mating experiments, the control group consisted of male offspring from the untreated dams. All of the control responses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were also not monitored. Animal husbandry details were reported in HERO ID 674171. No differences between groups were noted in the current study.
Domain 4: Selective Reporting and Attrition				

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	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.
Domain 6: Outcome Measures and Results Display				

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HERO ID:	673565

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The endpoint (body weights of male offspring) was sensitive, but it is unclear why body weights were not measured throughout the study instead of singular measurements of final body weights. There were some differences/inconsistencies that could have impacted the study results. For example, some male offspring were sacrificed on PND 144 ± 7 days. It is unclear why all animals were not sacrificed on the same day, and it isn't specified which groups were sacrificed when. If, for example, the controls were sacrificed on PND 144, and high-dose animals were sacrificed on PND 151, this could have a significant impact on the body weight results and makes it difficult to interpret the results reported. The number of animals used was appropriate for statistical analysis.
	Metric 9:	Results presentation	High	Body weight data were quantitatively reported as means ± SE. Statistical methods were described and were adequate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225(1):64-74.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Developmental effects: number of live and dead pups, pup body weights, sex, general signs of toxicity, nipple retention, anogenital distance, age of testes decent, external examinations of reproductive organs for malformations, histopathology of the testes, liver and brain weights on PND1, liver, brain, testis and epididymis weights on PND 22.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (From GD 6)-F0- lactation (through PND 21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673567

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and some important information were provided. Dams (11-16 per group) were dosed via gavage with di-(2-ethylhexyl) phthalate (DEHP) from GD6 through PND 21. The source and lot number were provided. The purity was not specified in the study, but all of the DEHP products on the source website are analytical or HPLC grade with purities $\geq$ 98%. The test animals were gravid female Wistar rats (HsdCpb:Wu); the source was specified along with initial animal weights (at purchase; 200 $\pm$ 15g). The parity and age of the animals were not specified. HERO ID 674171 (which published the female data for this study) was referenced for animal husbandry conditions, were all reported along with the number of animals per cage, Endpoint evaluation methods were adequately described, and quantitative results were reported for most endpoints.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The referenced study HERO 674171 indicated dams were randomly assigned into groups, but the method of randomization was not specified. It is unknown whether animals were normalized for body weight. The current study also indicated that 1 or 2 male pups were randomly selected from each litter for sacrifice on PND1. The method of randomization also was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Low	The study indicated that anogenital distance was measured in a blinded manner and that initial histopathology was also done blinded and there was no secondary histopathological evaluation. Blinding was not required for other endpoints because they were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative control dams were administered a peanut oil vehicle. All of the control responses appeared to be appropriate. The study did not monitor potentially confounding factors in the treated dams such as food and water intake. The body weights of dams were monitored and reported in HERO ID 674171 and there were no significant changes that were suggestive of palatability issues. Animal husbandry details reported in the same referenced study were consistent across groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225(1):64-74.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Developmental effects: number of live and dead pups, pup body weights, sex, general signs of toxicity, nipple retention, anogenital distance, age of testes decent, external examinations of reproductive organs for malformations, histopathology of the testes, liver and brain weights on PND1, liver, brain, testis and epididymis weights on PND 22.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (From GD 6)-F0- lactation (through PND 21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673567

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	HERO ID 674171 was referenced for information on dams. The number of litters per group varied (11-16), and this reference indicates this is also the number of dams. It is not clear why there are such differences between groups (e.g., if a common number was intended, but some dams died, or turned out not to be gravid). The study also did not report the total number of pups born, and viability between birth and PND22 was not reported. The number of pups sampled for different endpoints varied making it difficult to know whether there was evidence of attrition, or if the data were selectively reported. On PND 22 at the high dose, liver, brain, and epididymis weights were only obtained from 13 animals (7 litters), even though body weights were measured in 15 pups from 8 litters. The study authors did not describe the reasoning for the difference. Qualitative or quantitative data were reported for all of the specified outcomes and endpoints.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The study did not report the test substance purity; however, the commercial source (Sigma Aldrich) only sells DEHP products with purities $\geq$ 98%, and all are analytical or HPLC grade and provide data sheets and certifications of analysis for each product. The concentrations of the doses used in this study were verified by GCMS, although the study did not specify whether the analytical measurements were close to nominal, and it is presumed that the doses reported were nominal. The authors also did not report whether, or how often doses were adjusted to account for dam weight gain, and it is unknown at what point the analytical measurement were conducted (e.g., only once, or with each preparation etc). Although it was specified that a peanut oil vehicle was used, no information on preparation (e.g., frequency and timing), or on storage conditions were provided. The doses used in the study were adequately justified by the study authors. The lowest dose was similar to the estimated median daily intake of the general German population, and the highest dose was known to induce adverse reproductive effects in male offspring without causing over maternal toxicity. Gavage is an appropriate route of exposure for this chemical, and a consistent gavage volume of 5 mL/kg bw was used across groups. The missing information on frequency of dose preparations and details for frequency of adjustments for maternal body weights leads to some ambiguity about the precision of dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency, and duration of exposure (daily from GD 6 to lactation day 21, or weaning, was appropriate and sensitive for the purposes of the study. The time of day of dosing was not specified, but this is not expected to have a substantial impact on the interpretation of the study results.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225(1):64-74.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Developmental effects: number of live and dead pups, pup body weights, sex, general signs of toxicity, nipple retention, anogenital distance, age of testes decent, external examinations of reproductive organs for malformations, histopathology of the testes, liver and brain weights on PND1, liver, brain, testis and epididymis weights on PND 22.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (From GD 6)-F0- lactation (through PND 21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	673567

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	The endpoints were sensitive and specific for the outcomes of interest and were consistent with the purpose of the study which was to evaluate the sexual development of male offspring from birth to puberty, with a focus on androgen-sensitive endpoints. Animals in each group were consistently sacrificed on the same day. The sample sizes were appropriate and sufficient for performing statistical analysis and NOAEL and LOAEL values were determined, although histopathology was only performed on 4-6 pups per dose, which may have reduced the statistical power for that endpoint since no significant changes were observed. The authors provided justification for the doses chosen (based on the median daily intake of the general German population and doses previously shown to induce adverse effects in male offspring).
	Metric 9:	Results presentation	Medium	Data for all endpoints were presented quantitatively as means ± SE, or as incidences (histopathology), which included measures of severity. The methods of statistical analysis were described and were adequate. It does not appear that histopathology data were statistically analyzed, but sufficient data are provided for an independent analysis.

Additional Comments: This study briefly mentions that there were no effects on maternal weight gain, and there was no general toxicity in dams and refers the reader to the sister publication for this study. These data are quantitatively (weight gain) reported in HERO ID 674171; therefore, maternal data were not evaluated here.

Overall Quality Determination Medium

Study Citation:	Culty, M., Thuillier, R., Li, W., Wang, Y., Martinez-Arguelles, D., Benjamin, C., Triantafilou, K., Zirkin, B., Papadopoulos, V. (2008). In utero exposure to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat. Biology of Reproduction 78(6):1018-1028.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testosterone production in ex vivo fetal organ cultures.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14 - parturition)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	698207

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information was reported. Reported information included information on the test substance (name and source), the test model (species, strain, sex, and source), animal husbandry details (photoperiod, food and water availability), exposure methods, experimental design, endpoint evaluations, and presentation of results.Missing information included the purity of the test substance, test animal age, initial body weights, parity, additional animal husbandry details (temperature, humidity, number of animals per cage), and the number of dams per treatment group.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No details on the allocation of dams into study groups or on the selection of fetuses for outcome analysis were provided.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcome was measured using a standard laboratory kit.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. Consistency of other potentially confounding factors (e.g., most animal husbandry conditions, body weights, food or water intake, or gavage volume) were not reported. It is unclear whether the study took measures to minimize the exposure to other plasticizers which could influence the study results. No information on cage type or other materials used in the study were provided.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The total number of dams included in each test group was not specified. The data relevant to the endpoint of interest was derived from n = 3 litters. The study did not report endpoints that would allow for the determination of possible attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Culty, M., Thuillier, R., Li, W., Wang, Y., Martinez-Arguelles, D., Benjamin, C., Triantafilou, K., Zirkin, B., Papadopoulos, V. (2008). In utero exposure to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat. Biology of Reproduction 78(6):1018-1028.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testosterone production in ex vivo fetal organ cultures.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14 - parturition)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	698207

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma) was reported. The purity was not specified, and no certificate of analysis was provided in the study report. Purity and certificates of analysis would have been available on the supplier's website and the time of purchase. There is no indication that the test substance was verified by the performing laboratory. Animals were dosed via gavage in corn oil and the gavage volume was not reported. No details on the preparation, storage, or stability of the test solutions were provided. The study included 4 dose groups plus a control. Doses were reported in mg/kg-day and were adjusted daily based dam body weights, although the frequency of measurements was not specified. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD14 until parturition. This exposure covers the period of post-implantation embryonic development and the critical windows of organogenesis and male sexual differentiation.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	There are no major concerns regarding the specificity and validity of the protocol for measuring testosterone production; however, some details were missing. Testes (1 or 2 not specified) from GD20 fetuses (number not specified) from 3 litters per dose were cultured ex vivo for 1 or 2 days, or for 3 days with or without the addition of hCG. Testosterone production was measured using an ELISA assay. The dose spacing did not allow for the determination of a NOAEL for this endpoint. The test species and strain were appropriate for the study type.
	Metric 9:	Results presentation	High	Results were reported in a figure (bar graph) showing means ± SEM. Statistical significance and sample size (n=3 litters) were shown. The litter was used as the experimental unit. Individual animal data were not provided.

Additional Comments: Only fetal testosterone was evaluated for data quality. Testosterone levels in neonates or adult offspring were not evaluated.

Overall Quality Determination — Low

Study Citation:	Gray, L. E., Jr, Lambright, C. S., Conley, J. M., Evans, N., Furr, J. R., Hannas, B. R., Wilson, V. S., Sampson, H., Foster, D., P.M. (2021). Genomic and Hormonal Biomarkers of Phthalate-Induced Male Rat Reproductive Developmental Toxicity Part II: A Targeted RT-qPCR Array Approach That Defines a Unique Adverse Outcome Pathway. Toxicological Sciences 182(2):195-214.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Fetal testosterone production ex vivo
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-GD18)
Species:	Rat-Other (Crl:(CD)SD)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	9419406 Linked HERO ID(s): 9419406, 12162058

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information is reported. The test chemical was identified by name and CASRN. The source, lot, catalogue number, and purity are provided in a supplemental file by Fur et al. (2014). Other reported information includes test animal details (species, strain, source, age, initial body weights, and parity), animal husbandry details (number per cage, food and water availability, photoperiod, temperature, and humidity), exposure methods, experimental design, endpoint evaluations, and presentation of results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The authors stated that pregnant dams were randomly assigned to treatment groups on GD14 in a manner that provided each group with similar means and variances in body weight. The method of randomization was not specified.
	Metric 3:	Observational Bias / Blinding Changes	Medium	The paper did not indicate that whether investigators were blinded during outcome assessment. However, the outcome of interest was measured using standard laboratory kits.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Vehicle (laboratory-grade corn oil) and gavage volume were the same in the control and treatment groups. Animals were housed individually. The study did not specify whether measures were taken to reduce the potential for exposure to plasticizers, which could influence study results in a study focused on assessing the potential for endocrine disruption. Municipal drinking water was tested monthly for Pseudomonas and every 4 months for a suite of chemicals including pesticides and heavy metals. However, the materials used to dispense water to animals were not specified and it was not reported whether food was tested for phthalate contamination. Animals were housed in polycarbonate rather than metal cages. The experimental conditions described provided no indication of different practices across treatment groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	Quantitative data for the endpoint of interest were provided and all of the litters were accounted for. There is no evidence suggesting attrition or selective reporting.

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Study Citation:	Gray, L. E., Jr, Lambright, C. S., Conley, J. M., Evans, N., Furr, J. R., Hannas, B. R., Wilson, V. S., Sampson, H., Foster, D., P.M. (2021). Genomic and Hormonal Biomarkers of Phthalate-Induced Male Rat Reproductive Developmental Toxicity Part II: A Targeted RT-qPCR Array Approach That Defines a Unique Adverse Outcome Pathway. Toxicological Sciences 182(2):195-214.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Fetal testosterone production ex vivo
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD14-GD18)
Species:	Rat-Other (Crl:(CD)SD)-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	9419406 Linked HERO ID(s): 9419406, 12162058

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance source, catalogue number, lot number, and purity (>99%) was reported (Furr et al. 2014). The test substance was not analytically verified by the performing laboratory. No details of preparation or storage of the test solutions were provided. The doses were clearly reported and were adjusted daily based on dam body weights. The gavage volume (2.5 mL/kg) was appropriate. Concentrations of the test substance in the dosing solutions was not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Pregnant dams were dosed daily from GD14-GD18. The authors reported this as a critical period of sexual differentiation. This paper was a continuation of a previous publication (Furr et al. 2014) and maintained the same exposure details.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No concerns regarding the specificity and validity of the protocols and measures were identified. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. Samples were incubated individually for 3 hours. Measurements were collected from 1 testis/male from 3 males/litter from 3-4 litters.
	Metric 9:	Results presentation	High	Results for testosterone production are shown in Figure 2. The figure does not specify the sample size and is reported as a % of control so lacks measures of variance. However, raw data are available in the supplemental files. There are no notable concerns about the way the results are analyzed.

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

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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl 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.

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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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments
Domain 4: Selective Reporting and Attrition			
	Metric 5: Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from n = 3 controls and n = 4 for all other dose groups. However, the sample sizes for T production were from n = 6, 3, 3, 6, 4, 4, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.
Domain 5: Exposure Methods Sensitivity			
	Metric 6: Chemical administration and characterization	Medium	The purity or grade of the test substance was not reported but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure the concentrations in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.
	Metric 7: Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. 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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl 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. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. One testis each was dissected from the first 3 male fetuses/litter. The remaining testes were pooled to evaluate the expression of insl3, StAR, and Cyp11a. It is not clear whether the individual testes used in the testosterone assay were left or right, so differential/bilateral effects are not evaluated. Sample size is small (n=3 dams/dose group), but was validated by the authors to have sufficient statistical power to evaluate changes in fetal 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	High	Data were presented as means ± SE. The "n" is assumed to be the number of litters assessed. Statistical analysis was performed by authors and was appropriate.

Additional Comments: None

Overall 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-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl 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-Male Reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from n = 3 controls and n = 4 for all other dose groups. However, the sample sizes for T production were from n = 6, 3, 3, 6, 4, 4, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The purity or grade of the test substance was not reported but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure the concentrations in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure frequency, timing and duration were appropriate for the study's aim. 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 endpoints evaluated were sensitive to outcomes of interest. No concerns regarding the specificity of the protocols and measures were identified. qPCR samples were run in duplicate only, and it doesn't appear that there were any independent experimental replicates. It is not clear that cDNA levels were measured. An RNA to cDNA ratio of 1:1 was assumed. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. One testis each was dissected from the first 3 male fetuses/litter. The remaining testes were pooled to evaluate the expression of insl3, StAR, and Cyp11a. It is not clear whether the individual testes used in the testosterone assay were left or right, so differential/bilateral effects are not evaluated. Sample size is small (n=3 dams/dose group), but was validated by the authors to have sufficient statistical power to evaluate changes in fetal testosterone production, although authors stated that changes less than 20-25% may not be consistently detected (see Furr et al. 2014 [2510906]).

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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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Data were presented as means ± SE. The "n" is assumed to be the number of litters assessed. Statistical analysis was performed by authors and was appropriate.

Additional Comments: None

Overall 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)-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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl 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.
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)-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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from n = 3 controls and n = 4 for all other dose groups. However, the sample sizes for T production were from n = 6, 3, 3, 6, 4, 4, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.
Domain 5: Exposure 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 but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure from GD 14-18 occurs at the end of the critical window of organogenesis and does not include pre-mating or early gestational stages, so may be less sensitive for evaluating maternal effects and effects on fetal survival and growth.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods did not specify that dams were observed for mortality or clinical signs and no results for these endpoints were reported for animals treated with DEHP; however, results for these endpoints were reported for animals treated with other chemicals (e.g., DINP, DIBP) and therefore, it is assumed that these endpoints were evaluated in all animals in the study. No details were provided on the frequency of observations.
	Metric 9:	Results presentation	Uninformative	No results for mortality or clinical observations were provided in the text.

Additional Comments: None

Overall Quality Determination — Uninformative

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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)-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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from n = 3 controls and n = 4 for all other dose groups. However, the sample sizes for T production were from n = 6, 3, 3, 6, 4, 4, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.
Domain 5: Exposure 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 but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.
	Metric 7:	Exposure timing, frequency, and duration	High	Exposure from GD 14-18 occurs at the end of the critical window of organogenesis and does not include pre-mating or early gestational stages, so may be less sensitive for evaluating maternal effects and effects on fetal survival and growth.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods did not specify that dams were observed for mortality or clinical signs and no results for these endpoints were reported for animals treated with DEHP; however, results for these endpoints were reported for animals treated with other chemicals (e.g., DINP, DIBP) and therefore, it is assumed that these endpoints were evaluated in all animals in the study. No details were provided on the frequency of observations.
	Metric 9:	Results presentation	Uninformative	No results for mortality or clinical observations were provided in the text.

Additional Comments: None

Overall Quality Determination — Uninformative

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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)-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- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments

Study Citation:	Hannas, B. R., Lambright, C. S., Furr, J., Howdeshell, K. L., Wilson, V. S., Gray, L. E. (2011). Dose-response assessment of fetal testosterone production and gene 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
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl 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.
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
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from n = 3 controls and n = 4 for all other dose groups. However, the sample sizes for T production were from n = 6, 3, 3, 6, 4, 4, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.
Domain 5: Exposure 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 but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure frequency, timing and duration were appropriate for the study's aim. 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	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.

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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.
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Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	Body weight data is not reported for all dams (3 control and 4 in treatment groups). The body weight gain is shown graphically. Statistical analysis is performed by the study authors. Other data are reported as negative in the text.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	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.
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Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl 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.
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.
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Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	The methods state that there were 3-6 dams treated/group. It was not specified whether dams were observed for mortality, and no deaths were reported. The actual number of animals per group is unclear due to differences in sample sizes provided in the results. For example, data reporting maternal body weights are from n = 3 controls and n = 4 for all other dose groups. However, the sample sizes for T production were from n = 6, 3, 3, 6, 4, 4, and 3 litters in the 0, 100, 300, 500, 625, 750, and 874 mg/kg-day groups, respectively. Due to reporting a range for the number of animals per group, and the differences in sample sizes across endpoints, it is possible some animals were not included in the assessment for some reason (i.e. death, outlier, illness), or there was selective reporting for some endpoints.
Domain 5: Exposure 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 but Sigma's website indicates a purity of >98%. The test substance was not analytically verified by the performing laboratory; however, Sigma generally will supply certificates of analysis upon request. The study did not measure concentration in corn oil or report if doses were prepared fresh. No details on homogeneity or stability were provided. Dams were dosed daily by oral gavage. It is not reported whether doses were adjusted daily based on maternal body weight. The gavage volume (2.5 mL/kg) was appropriate.
	Metric 7:	Exposure timing, frequency, and duration	Medium	The exposure frequency, timing and duration were appropriate for the study's aim. 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	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.

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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
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 14-18)
Species:	Rat-Other (Sprague-Dawley- Charles River)-Female-Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	788239

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Body weight data is not reported for all dams (3 control and 4 in treatment groups). The body weight gain is shown graphically. Statistical analysis is performed by the study authors. Other data are reported as negative in the text.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Hellwig, J., 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and 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:	Diethylhexyl 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and 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:	Diethylhexyl 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and 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:	Diethylhexyl 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:	Diethylhexyl Phthalate- Parent compound
HERO ID:	674193 Linked HERO ID(s): 674193, 1325530

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test materials were clearly identified by names and CASRNs. The source and 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:	Diethylhexyl 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:	Howdeshell, K. L., Wilson, V. S., Furr, J., Lambright, C. R., Rider, C. V., Blystone, C. R., Hotchkiss, A. K., Gray, L. E., Jr (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicological Sciences 105(1):153-165.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Male reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 8-18)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	675206

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	Good. All critical and most important information was reported. Reported information included information on the test substance (name, source, purity), the test model (species, strain, sex, and source, animal husbandry details (animals per cage, photoperiod, temperature, food and water availability), exposure methods, experimental design, endpoint evaluations, and presentation of results.Missing information included the test animal age, initial body weights, parity, and humidity.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Adequate. Authors stated pregnant dams were assigned to treatment groups on GD 8 in a manner that provided similar mean body weight per treatment group prior to dosing. It is not clear whether this was done randomly, but this description indicates that 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. Animals were housed individually. The study did not specify whether measures were taken to reduce the potential for exposure to plasticizers, which could influence study results in a study focused on assessing the potential for endocrine disruption. Water was tested monthly for Pseudomonas and every 4 months for a suite of chemicals including pesticides and heavy metals. However, the materials used to dispense water to animals was not specified and it was not reported whether food was tested for phthalate contamination. Animals were housed in polycarbonate rather than metal cages. The experimental conditions described provided no indication of different practices across treatment groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Howdeshell, K. L., Wilson, V. S., Furr, J., Lambright, C. R., Rider, C. V., Blystone, C. R., Hotchkiss, A. K., Gray, L. E., Jr (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicological Sciences 105(1):153-165.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Male reproductive - testosterone
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 8-18)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	675206

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Adequate. All endpoints described in methods were reported qualitatively or quantitatively. All dams/litters are accounted for in the maternal weight gain, litter size, resorptions, and fetal mortality data (Table 2). A small number of dams died or were removed from the study due to dosing errors, as described in the text. The numbers of fetuses and litters used to determine testicular testosterone production (Table 6) were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Adequate. Source of chemical was reported (Sigma-Aldrich, who reported a purity of 99%). There was no indication that the authors independently verified the concentration or stability of the test chemical. The vehicle (laboratory grade corn oil) was also purchased from Sigma-Aldrich. Rat dams were weighed daily during the dosing period to administer the dose per kg body weight.
	Metric 7:	Exposure timing, frequency, and duration	High	All outcomes: Good. Pregnant dams were dosed daily with DIBP from GD 8-18. This exposure covers the period of post-implantation embryonic development, including the critical windows of organogenesis and male sexual differentiation.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Good. There are no concerns regarding the specificity and validity of the protocols and measures were identified. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. Testes from all of the males were incubated for fetal testicular hormone production. The methods stated that both testes were dissected and incubated individually. Results were obtained from the following sample sizes per dose (fetuses, litters): 0 (24, 4), 100 (26, 4), 300 (27, 4), 600 (29, 4), and 900 (20, 4) mg/kg-day. These sample sizes are considered to be adequate.
	Metric 9:	Results presentation	High	All outcomes: Good. There are no notable concerns about the way the results are analyzed or presented.

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

Overall Quality Determination — High

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testis, epididymis, prostate, seminal vesicles, ovaries including the oviducts, uterus); Histopathology (testis, epididymis, prostate, seminal vesicles, ovary, oviduct, uterus, and vagina);Sperm parameters (percent of motile sperm, concentration and percentage of abnormal sperm);Mating and fertility indices (copulatory plug, number of fertile pairs/number cohabitated, litter/pair);F1: live pup body weight, sex ratio, proportion of pups born alive, number of live pups/litter
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Organ weight (testis, epididymis, prostate, seminal vesicles, ovaries including the oviducts, uterus); Histopathology (testis, epididymis, prostate, seminal vesicles, ovary, oviduct, uterus, and vagina);Sperm parameters (percent of motile sperm, concentration and percentage of abnormal sperm);Mating and fertility indices (copulatory plug, number of fertile pairs/number cohabitated, litter/pair);F1: live pup body weight, sex ratio, proportion of pups born alive, number of live pups/litter
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				

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Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
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Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.
	Metric 9:	Results presentation	Low	Results were described in the text and data were presented in tables as means ± standard error. Statistical analysis methods were reported. The study used the pup instead of the litter as the unit of statistical analysis, this has the potential to overestimate statistical significance of experimental findings (Dishaw et al. 2020). Individual animal data was not reported.

Additional Comments: None

Overall Quality Determination — Low

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Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.

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Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.

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Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight
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Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
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Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
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Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
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Domain 4: Selective Reporting and Attrition				

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	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.

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Domain	Metric	Rating	Comments
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Additional Comments: None

Overall Quality Determination Medium

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	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were reported and considered sensitive for most endpoints. Necropsy and histopathology were performed only on the highest dose group and control group.

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Hepatic/Liver-Liver weight-Neurological/Behavioral-Brain weight-Other (please specify below) (Endocrine)-Pituitary weight
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Data for mortality and organ weights were fully reported. Organ weights were reported as means +/- SE along with number of animals. Statistical methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies for assessing clinical signs were not reported (frequency, detailed or cage-side observations).

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	Clinical signs were reported as negative in the text.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies for assessing clinical signs were not reported (frequency, detailed or cage-side observations).

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Other (please specify below) (Clinical signs)-Clinical signs of toxicity
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	Clinical signs were reported as negative in the text.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (CASRN was not provided). The source and purity (>99%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins. Initial body weights were not reported. Husbandry conditions (temperature, light cycle, number of animals/cage) were reported; humidity was not reported. Cage type and bedding type were reported. Food and water were provided ad libitum. Route of exposure was reported. Doses were reported as % in food. Target concentrations were reported, and analytical concentrations were reportedly within 86-107% of target concentration for DEHP and 96- 107% for DBP. Duration of exposure was reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were allocated to groups by stratified randomization procedure based on body weights.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, endpoints evaluated were either not subjective in nature or consisted of clinical signs or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Body weight and food intake were not sufficiently reported in this dietary exposure study to determine confounding. Food intake was reported to be similar between the groups, however the study did not distinguish between when mice were pregnant vs non-pregnant or male/female difference. A negative control group was included (Purina certified chow) and responses were appropriate. Housing conditions that were specified seemed to be consistent across groups (humidity was not reported). The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Body weights of maternal animals at initiation or during the study were not reported. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for most outcomes in tabular form or within text. Not all animals were accounted for in results. For example, in Table 12 data for 35-36 control males are reported (out of 40) and Table 13, data for 14-15 treated females are reported (out of 18). No explanation is provided for why data from these animals were not included. Methods stated that pups were assessed for sex ratio but results were not reported for this endpoint. Overall, the omissions and attrition are not explained but they are not expected to significantly impact the interpretation of the results. Sample size was stated in the methods and the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source of the test substance was reported. The purity of the test substance was independently verified by chromatography and spectrometric analysis to be 99%. Test substance formulations were mixed separately every week, stored at 4°C until used, and tested every 6 weeks by Midwest Research Institute for referee analysis. The compound was reportedly stable in room air and light for 7 days. Target test concentrations were reported (%); data in mg/kg/day was not provided. Feed intake and body weights were not fully reported. Study authors stated that because pairs were cohabiting that it was not possible to accurately determine relative dose in mg/kg.
	Metric 7:	Exposure timing, frequency, and duration	Low	Doses were selected to ensure no severe systemic toxicity at the highest dose and lesser or no toxicity at the two lower doses; however, it was unclear if this was based on previous toxicity studies. Animals were exposed for a 7-day premating period, paired, and then exposed an additional 98 days. Pairs were then separated and exposed for 21 days. Exposure was consistent across study groups. Groups were treated concurrently. The OECD 422 and 443 Guidelines which examine similar endpoints suggest a premating period of 14 days as opposed to 7 days. This shortened pre-mating period may not have allowed females to have completed enough oestrous cycles to sufficiently detect adverse effects or for males to have adequate time for the maturing of spermatozoa.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified. The number of animals/group was appropriate as group sizes were large enough to yield at least 20 pregnant females/group and sufficient for statistical analysis. Three treated groups and one control group were utilized which was appropriate. The OECD guidelines recommend using doses that are two to four-fold increase intervals apart. While the study authors used doses with ten-fold intervals, excessive death was not observed at the highest dose and adverse effects were not observed at the lowest dose. Outcome methodologies were not adequately reported. Timing of measurements for food intake and body weights were not reported.

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Study Citation:	Lamb, J., Chapin, R., Teague, J., Lawton, A., Reel, J. (1987). Reproductive effects of four phthalic acid esters in the mouse. Toxicology and Applied Pharmacology 88(2):255-269.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (7 days)-F0- mating (98 days)-F0 - gestation (21)-F0- premating (7 days)-F0- mating (98 days)
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	61566

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Necropsy body weights were reported as means +/- SE for control and high dose groups. Statistical analysis was reported and appropriate. Body weights at 1 and 13 weeks are not fully reported (SE not included, only males reported, only high-dose and control group reported). Food intake was reported in text as a range for all groups combined.

Additional Comments: None

Overall Quality Determination Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information was reported. Reported information included information on the test substance (name and source), the test model (species, strain, sex, and source), exposure methods, experimental design, endpoint evaluations, and presentation of results.Missing information included the purity of the test substance, test animal age, initial body weights, parity, and all animal husbandry details.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No details on the allocation of dams into study groups or on the selection of pups for outcome analysis were provided.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcomes were simple measures or were measured or quantified using standard laboratory kits.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	A negative corn oil control group was included. There were no differences in dam or pup body weights and gavage volumes were consistent across groups. Consistency of other potentially confounding factors (e.g., animal husbandry conditions) was not reported. It is unclear whether the study took measures to minimize the exposure to other plasticizers which could influence the study results for this health outcome.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	All dams were accounted for in the study and data for the endpoint of interest were reported. However, there is a range of sample sizes for each endpoint that are not justified by the authors, and/or the sample sizes for the endpoints of interest are confusing (see Metric 6.1). It is unclear if data from any animals were excluded.
Domain 5: Exposure Methods Sensitivity				

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma) was reported, but the purity was not specified. No certificate of analysis was provided, and there is no indication that the test substance was verified by the performing laboratory. Animals were dosed via gavage in corn oil and the gavage volume (1mL/kg) was appropriate. No details on the preparation, storage, or stability of the test solutions were provided. Doses were reported in mg/kg-day. It is not specified whether doses were adjusted daily based on measured body weights. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD2-20. This exposure covers the period of pre-implantation embryonic development, and the critical windows of organogenesis and male sexual differentiation.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	Limited methodological details were provided. Testicular steroids were extracted from "the testes of control and DEHP-exposed pups (n = 10 control; n = 12 DEHP)" on GD21 and testosterone concentrations were measured using an ELISA assay. It was not specified whether this was the number of pups per litter, or if one or both testes were evaluated. The number of animals (dams) per group were n = 6 for controls and low and mid-dose groups, and n = 9 for the high dose. No additional methodological details were provided. Two studies (Akingbemi et al., 2001 and 2004) were cited for additional methodological details and were reviewed for this evaluation; these studies did not provide many useful relevant methodological details as they also cited other studies for methodological details. The sample sizes for other endpoints were also a range (e.g., 10-11, or 8-10), but were sufficient for conducting statistical analysis. Some methodological details for gene expression analysis were provided in supporting information files. There are no concerns for the test animals selected. There was no clear justification for the dose selection and spacing, although the doses had been used in previous studies. A NOAEL could not be determined; however, the purpose of the study was more to characterize and understand the mechanisms of already known effects, rather than to identify a NOAEL.
	Metric 9:	Results presentation	Low	Quantitative data were provided for all of the specified endpoints as incidences or as means ± SEM across all dose groups. Some statistical methods were described, but there is no indication that the litter was used as the experimental unit for any endpoint. Statistical significance is clearly shown. Individual animal data were not provided.

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

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Low**	

Study Citation:	Lin, H., Ge, R., Chen, G., Hu, G., Dong, L., Lian, Q., Hardy, D., Sottas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in fetal Leydig cell aggregation after exposure to phthalate in utero. Proceedings of the National Academy of Sciences of the United States of America 105(20):7218-7222.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Dam body weights
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD2-20)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	698185

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information was reported. Reported information included information on the test substance (name and source), the test model (species, strain, sex, and source), exposure methods, experimental design, endpoint evaluations, and presentation of results.Missing information included the purity of the test substance, test animal age, initial body weights, parity, and all animal husbandry details.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No details on the allocation of dams into study groups were provided.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcomes were simple measures (dam body weights)
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included. There were no differences in dam body weights and gavage volumes were consistent across groups. Consistency of other potentially confounding factors (e.g., animal husbandry conditions) was not reported. It is unclear whether the study took measures to minimize the exposure to other plasticizers.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All dams were accounted for in the study and data for the endpoint of interest were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test material source (Sigma) was reported, but the purity was not specified. No certificate of analysis was provided, and there is no indication that the test substance was verified by the performing laboratory. Animals were dosed via gavage in corn oil and the gavage volume (1mL/kg) was appropriate. No details on the preparation, storage, or stability of the test solutions were provided. Doses were reported in mg/kg-day. It is not specified whether doses were adjusted daily based on measured body weights. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD2-20. This exposure covers the period of pre-implantation embryonic development, and the critical windows of organogenesis and male sexual differentiation, which was the focus of the study.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Lin, H., Ge, R., Chen, G., Hu, G., Dong, L., Lian, Q., Hardy, D., Sottas, C., Li, X., Hardy, M. (2008). Involvement of testicular growth factors in fetal Leydig cell aggregation after exposure to phthalate in utero. Proceedings of the National Academy of Sciences of the United States of America 105(20):7218-7222.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Dam body weights
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD2-20)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	698185

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Medium	Limited methodological details were provided. The data figure legend indicates that dams were weighed before and after exposure, but the exact timing of the measurements was not specified. This is not expected to have a significant impact on the study results. The sample sizes were specified (all animals were weighed). There are no concerns over the test animals selected for the study. A wide dose range was used for this study. There was no clear justification for the dose selection and spacing, although the doses had been used in previous studies.
	Metric 9:	Results presentation	High	Quantitative data were provided as means ± SEM across all dose groups. Statistical methods were described and were appropriate. Individual animal data were not provided.

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

Overall Quality Determination — Medium

Study Citation:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weights (GD 12 and GD 20 and GD 21.5)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697737

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical information is reported. Test substance is identified by name, and the supplier is reported. The test substance purity was not reported. Test animal species, strain, sex, and commercial source were reported. Starting body weights are reported in the results (table 1). Test animal starting age was not reported but described as “adult” and animal husbandry conditions were not described in any detail, with the authors merely stating that ”all animal procedures were performed in accordance with the policies of the Rockefeller University’s Animal Care and Use Committee”. These deficiencies could significantly impact the quality of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors did not explain how animals were allocated into groups.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were described, but endpoints of interest are unlikely to be significantly influenced by observational bias as they were not subjective.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An appropriate negative vehicle control is included and there was no response in the control group. Not all information was reported to evaluate confounding (food/water consumption, presence of EDCs in bedding materials). The authors did not discuss whether measures were taken to reduce exposure to plasticizers but this is not expected to significantly impact the endpoint described.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weights (GD 12 and GD 20 and GD 21.5)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697737

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	The authors do not report whether or not any animals died over the course of the study, and do not report any health outcomes unrelated to the exposure. Not all animals appear to be accounted for in the results, many endpoints report a sample size of N=4-6 or 5-6 per group, despite having 11-13 dams in each group. In Table 2, it seems to be reported that 35-39 pups were in each group, however only 6-13 pups were listed for organ weight data on PND49. These omissions are not explained, and it isn't clear if a large number of pups died during early post-natal development, or if the authors selectively measured endpoints in specific animals. Animals that were not pregnant or did not deliver were excluded. The authors did explain that 1-2 dams from the 10 and 750 mg/kg/day group did not give birth to any pups, but this does not account for the incomplete sampling presented for most outcomes. The significantly smaller sample size reported for most endpoints is a significant concern for data quality. All prespecified outcomes were reported in the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The purity of the test substance was not reported and was not analytically determined by the authors. Test substance preparation was described, but storage conditions were omitted. Gavage volume was reported and is 1 mL/kg, which is appropriate. Doses were not analytically confirmed but are reported nominally in mg/kg units. As the purity of the test substance is not reported, these deficiencies are expected to significantly impact the quality of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure is sensitive for endpoints of interest, and covers the window of sensitivity (leydig cell marker detectable beginning GD12.5) for developmental male reproductive health effects.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	There are no concerns regarding the test animal species and strains, though there are some concerns regarding the sample size. Animal numbers used (11-13) were fewer than generally recommended by guidance (20) and outcome sample sizes were incomplete and varied between different endpoints without justification. It is not clear if the authors measured every outcome in every animal due to the inconsistent sample sizes. Outcome assessment methods are described completely and are appropriate. The authors justified their dose-range based off of results seen in previous studies, though the dose range could benefit from using an additional lower dose with the goal of determining a NOAEL.

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Study Citation:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Maternal body weights (GD 12 and GD 20 and GD 21.5)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697737

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Low	The authors do not specifically report whether or not the litter is the unit of sampling. Reported data (such as in Table 2) suggests that all reported results are from all of the available pups. Most results are reported quantitatively with measures of variance in tables and figures, with some negative data reported qualitatively. Statistical analysis appears to be appropriate and is described in the methods section.

Additional Comments: None

Overall Quality Determination Low

Study Citation:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Birth rate in dams and number of pups per dam. Endpoints assessed in pups: male:female ratio, anogenital distance (AGD) at PND2, body weight at PND35 and 49, testes and prostate weight at PND49, Leydig cell histopathology (average, median and maximum number of cells per cluster), testes mRNA expression, protein expression and enzyme activity, serum testosterone levels (PND 21 and 49).
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697737

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical information is reported. Test substance is identified by name, and the supplier is reported. The test substance purity was not reported. Test animal species, strain, sex, and commercial source were reported. Starting body weights are reported in the results (table 1). Test animal starting age was not reported but described as “adult” and animal husbandry conditions were not described in any detail, with the authors merely stating that ”all animal procedures were performed in accordance with the policies of the Rockefeller University’s Animal Care and Use Committee”. These deficiencies could significantly impact the quality of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The authors did not explain how animals were allocated into groups.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were described, but endpoints of interest are unlikely to be significantly influenced by observational bias as they were not subjective.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	An appropriate negative vehicle control is included and there was no response in the control group. Not all information was reported to evaluate confounding (food/water consumption, presence of EDCs in bedding materials) and the authors did not discuss whether measures were taken to reduce exposure to plasticizers. This may have a significant impact on the study results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	The authors do not report whether or not any animals died over the course of the study, and do not report any health outcomes unrelated to the exposure. Not all animals appear to be accounted for in the results, many endpoints report a sample size of N=4-6 or 5-6 per group, despite having 11-13 dams in each group. In Table 2, it seems to be reported that 35-39 pups were in each group, however only 6-13 pups were listed for organ weight data on PND49. These omissions are not explained, and it isn’t clear if a large number of pups died during early post-natal development, or if the authors selectively measured endpoints in specific animals. Animals that were not pregnant or did not deliver were excluded. The authors did explain that 1-2 dams from the 10 and 750 mg/kg/day group did not give birth to any pups, but this does not account for the incomplete sampling presented for most outcomes. The significantly smaller sample size reported for most endpoints is a significant concern for data quality. All prespecified outcomes were reported in the results.

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Study Citation:	Lin, H., Lian, Q., Hu, G., Jin, Y., Zhang, Y., Hardy, D., Chen, G., Lu, Z., Sottas, C., Hardy, M., Ge, R. (2009). In utero and lactational exposures to diethylhexyl-phthalate affect two populations of Leydig cells in male Long-Evans rats. Biology of Reproduction 80(5):882-888.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Birth rate in dams and number of pups per dam. Endpoints assessed in pups: male:female ratio, anogenital distance (AGD) at PND2, body weight at PND35 and 49, testes and prostate weight at PND49, Leydig cell histopathology (average, median and maximum number of cells per cluster), testes mRNA expression, protein expression and enzyme activity, serum testosterone levels (PND 21 and 49).
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12.5-GD20)-F0- lactation (PND 0-PND 21)
Species:	Rat-Long-Evans - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	697737

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The purity of the test substance was not reported and was not analytically determined by the authors. Test substance preparation was described, but storage conditions were omitted. Gavage volume was reported and is 1 mL/kg, which is appropriate. Doses were not analytically confirmed but are reported nominally in mg/kg units. As the purity of the test substance is not reported, these deficiencies are expected to significantly impact the quality of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure is sensitive for endpoints of interest, and covers the window of sensitivity (leydig cell marker detectable beginning GD12.5) for developmental male reproductive health effects.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	There are no concerns regarding the test animal species and strains, though there are some concerns regarding the sample size. Animal numbers used (11-13) were fewer than generally recommended by guidance (20) and outcome sample sizes were incomplete and varied between different endpoints without justification. It is not clear if the authors measured every outcome in every animal due to the inconsistent sample sizes. Outcome assessment methods are described completely and are appropriate. The authors justified their dose-range based off of results seen in previous studies, though the dose range could benefit from using an additional lower dose with the goal of determining a NOAEL.
	Metric 9:	Results presentation	Low	The authors do not specifically report whether or not the litter is the unit of sampling. Reported data (such as in Table 2) suggests that all reported results are from all of the available pups. Most results are reported quantitatively with measures of variance in tables and figures, with some negative data reported qualitatively. Statistical analysis appears to be appropriate and is described in the methods section.

Additional Comments: None

Overall Quality Determination — Low

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for modifying factors across groups were noted by the study authors. In addition, for this experiment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				

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

Domain	Metric		Rating	Comments
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.
Domain 6: Outcome Measures and Results Display				

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

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

Additional Comments: 1. DEHP Supp study in utero in rats

Overall Quality Determination — Low

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for modifying factors across groups were noted by the study authors. In addition, for this experiment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.

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

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.
Domain 6: Outcome Measures and Results Display				

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

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

Additional Comments: 1. DEHP Supp study in utero in rats

Overall Quality Determination — Low

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for modifying factors across groups were noted by the study authors. In addition, for this experiment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.

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

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.
Domain 6: Outcome Measures and Results Display				

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

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

Additional Comments: 1. DEHP Supp study in utero in rats

Overall Quality Determination — Low

Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical observations)-Clinical Observations
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for endpoint evaluations was provided. No other methods to control for modifying factors across groups were noted by the study authors. In addition, for this experiment, two female rats were paired with one male rat to form a breeding group. For each dose level, there were 5 breeding groups formed (10 female rats/group). However, for prenatal developmental toxicity studies (OECD guideline 414) it is recommended that females inseminated by the same male should be evenly distributed across the study groups. It is unclear if this occurred in this study. This could potentially substantially impact the interpretation of the results.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical observations)-Clinical Observations
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0 - gestation (20 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Quantitative or qualitative results were reported for most, but not all outcomes described in the methods (comments on clinical signs were not present in the results section). Overall, this is not expected to notably impact the interpretation of the results. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure were appropriate for the study type and endpoints of interest; however, it is unclear whether the duration of exposure was consistent; animals were dosed for "up to 20 days." The study text suggests groups of animals were sacrificed starting on GD17.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was an in utero developmental exposure study and dams were observed for clinical signs. The frequency of observations was reported, and it was specified that results were recorded "as needed." The test model, including the source and strain were appropriate for the evaluation of the endpoints. The sample size (10 pregnant females/group) was small, but sufficient for performing statistics.
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for clinical signs.

Additional Comments: 1. DEHP Supp study in utero in rats

Overall Quality Determination — Uninformative

Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The window of exposure (days 1-22 of lactation) differs from standard developmental studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	Quantitative data (mean ± SEM) were provided for the endpoints Day 0 litter weight, individual pup body weight (Day 7, 14, 21), pup absolute liver weight, palmitoyl-CoA oxidase activity in livers of pups, absolute liver weights of dams, palmitoyl-CoA oxidase activity in dam livers, and terminal body weights of dams. Statistical significance was provided for these endpoints. In addition, the study groups were clearly provided/indicated. It should be noted that the day of parturition and day of lactation varied between the exposure groups. As a result, two sets of analyses were performed for each day of evaluation. Data for the following endpoints: sex and number of pups and litter weights on LD0 and 1; number, sex, and individual pup body weights on LD4, were not provided.

Additional Comments: 2.DEHP Supp study lactational in rats

Overall Quality Determination — Low

Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The window of exposure (days 1-22 of lactation) differs from standard developmental studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	Quantitative data (mean ± SEM) were provided for the endpoints Day 0 litter weight, individual pup body weight (Day 7, 14, 21), pup absolute liver weight, palmitoyl-CoA oxidase activity in livers of pups, absolute liver weights of dams, palmitoyl-CoA oxidase activity in dam livers, and terminal body weights of dams. Statistical significance was provided for these endpoints. In addition, the study groups were clearly provided/indicated. It should be noted that the day of parturition and day of lactation varied between the exposure groups. As a result, two sets of analyses were performed for each day of evaluation. Data for the following endpoints: sex and number of pups and litter weights on LD0 and 1; number, sex, and individual pup body weights on LD4, were not provided.

Additional Comments: 2.DEHP Supp study lactational in rats

Overall Quality Determination — Low

Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The window of exposure (days 1-22 of lactation) differs from standard developmental studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-No. fetuses/breeding group, Litter weight; Gestation length, number of pups/litter, number of live pups/litter, percentage of live pups/litter, number of pups/sex/litter, Offspring clinical observations, mortality, feed consumption, histologic examinations on >30 organs/tissues, gross necropsy, offspring body weights, number of implantation sites, mating index, fertility index-Hepatic/Liver-Absolute liver weights of dams, palmitoyl-CoA oxidase activity of dams (Studies 1, 2, 3, and 4).Serum chemistry (ALP, ALT, total protein, albumin, total cholesterol, triglycerides, sorbitol dehydrogenase, bile acids, and glucose), Histopathology of liver (Studies 8 and 9). Absolute and relative liver weight (Studies 8, 9, 10, and 11).-Nutritional/Metabolic-Terminal body weights (Studies 1, 2, 3, and 4).Body weight, feed consumption (Studies 5, 6, 7, 8, 9, 10, 11, and 12).Body weight gain (Studies 5, 6, 7, and 12).
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
	Metric 9:	Results presentation	Medium	Quantitative data (mean ± SEM) were provided for the endpoints Day 0 litter weight, individual pup body weight (Day 7, 14, 21), pup absolute liver weight, palmitoyl-CoA oxidase activity in livers of pups, absolute liver weights of dams, palmitoyl-CoA oxidase activity in dam livers, and terminal body weights of dams. Statistical significance was provided for these endpoints. In addition, the study groups were clearly provided/indicated. It should be noted that the day of parturition and day of lactation varied between the exposure groups. As a result, two sets of analyses were performed for each day of evaluation. Data for the following endpoints: sex and number of pups and litter weights on LD0 and 1; number, sex, and individual pup body weights on LD4, were not provided.

Additional Comments: 2.DEHP Supp study lactational in rats

Overall Quality Determination — Low

Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical observations)-Clinical Observations
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
	Metric 1: Reporting Quality	Medium	All critical and most important information was reported in this study. The study included identification of the test substance (Di(2-ethylhexyl)phthalate), and source (Hatco Chemical Corporation); test animal characteristics (source, strain, sex, age); general animal husbandry conditions (temperature, humidity, diet, water availability, number of animals per cage); exposure methods (test substance source, purity, method of administration); experimental design (frequency of exposure, number of animals per study group, animal age during exposure); and endpoint evaluation methods (quantitative and qualitative). The study lacked the starting body weights of the dams and did not report parity. The missing information is not expected to significantly impact the study evaluation.
Domain 2: Selection and Performance			
	Metric 2: Allocation	Low	No information on the methods of allocation of animals into test groups or selection of animals for interim sacrifices was provided. No other methods to control for modifying factors across groups were noted by the study authors.
	Metric 3: Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported. However, the endpoints of interest were objective or simple measures.
Domain 3: Confounding / Variable Control			
	Metric 4: Confounding / Variable Control	Low	The study included a negative control group, which received un-dosed feed. A positive control group was not included and is not required. The study authors did not measure food consumption among the dams in a dietary study. It is unclear whether there were issues with palatability or if any differences in food consumption influenced the study results. Decreased body weights were observed. The authors did not specify whether measures were taken to reduce exposure to plasticizers and the test substance is a known endocrine disruptor.
Domain 4: Selective Reporting and Attrition			
	Metric 5: Selective Reporting and Attrition	Low	Quantitative or qualitative results were reported for only some outcomes described in the methods. The following results were not reported: Sex and number of pups and litter weights on LD0 and 1; number, sex, and individual body weights on LD4, or results of observations for clinical signs. The study methods reported dosing 12 female rats/group; however, female data were only reported for 6 females per group (and 18 for controls). The methods state there should have been data for 24 controls. There is no indication of animal attrition.

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Study Citation:	Marsman, D. S. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxicity Report Series, vol. 30 30:1-G5.
Health Outcome(s) and Reported Health Effect(s):	Other (please specify below) (Clinical observations)-Clinical Observations
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- lactation (21 days)
Species:	Rat-Fischer 344 - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	680063

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP-dosed feed. The purity (>99%) and storage conditions of the test substance were reported. The stability of the DEHP feed mixtures were tested at different temperatures using gas chromatography. It was found that DEHP mixtures were stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. The route and method of exposure were suited to the test substance. The study reported the concentrations of the test material as ppm in food. The authors do not report the calculated dose in mg/kg-animal/day. In addition, they do not provide sufficient information to independently calculate doses from concentrations in feed as they do not provide dam body weight at the start of or throughout the study period, or feed consumption. This uncertainty in the exposure characterization is expected to impact the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The window of exposure (days 1-22 of lactation) differs from standard developmental studies; however, the authors justified this exposure window which was appropriate for this study and the endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	This was a lactational exposure study. The outcome assessment methods were clearly described and were appropriate and sensitive for the outcomes of interest. The test model, including the source and strain were appropriate for the evaluation of the endpoints. Dose spacing was adequate for determination of NOAEL/LOAEL values. The sample sizes were sufficient for conducting statistical analysis.
	Metric 9:	Results presentation	Uninformative	Neither quantitative nor qualitative data were provided for clinical signs.

Additional Comments: 2.DEHP Supp study lactational in rats

Overall Quality Determination — Uninformative

Study Citation:	Martino-Andrade, A. J., Morais, R. N., Botelho, G. G., Muller, G., Grande, S. W., Carpentieri, G. B., Leao, G. M., Dalsenter, P. R. (2008). Coadministration of active phthalates results in disruption of foetal testicular function in rats. International Journal of Andrology 32(6):704-12.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular testosterone levels
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD13-GD21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	676281

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information was reported. Reported information included information on the test substance (name, CASRN, purity and source), the test model (species, strain, sex, and source), animal husbandry details (photoperiod, temperature, food and water availability), exposure methods, experimental design, endpoint evaluations, and presentation of results.Missing information included test animal age, initial body weights, parity, humidity, and number of animals per cage.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No details on the allocation of dams into study groups or on the selection of fetuses for outcome analysis were provided.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcome was measured using a standard laboratory kit.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative corn oil control group was included and gave the expected response. There were no differences in maternal body weights (fetal weights were not measured) and gavage volumes were consistent across groups. No differences in the animal husbandry parameters reported were noted. It is unclear whether the study took measures to minimize the exposure to other plasticizers (e.g., from cage, bedding, or water dispensing materials, or in food), which could influence the study results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The number of dams used in the study was reported as a range (6-9 per group). It was not specified if any of the dams used for this endpoint died. Data were reported for the endpoint of interest and the sample sizes for the from 6-8 (litters)/group. Based on the information provided, there is no evidence of selective reporting.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Martino-Andrade, A. J., Morais, R. N., Botelho, G. G., Muller, G., Grande, S. W., Carpentieri, G. B., Leao, G. M., Dalsenter, P. R. (2008). Coadministration of active phthalates results in disruption of foetal testicular function in rats. International Journal of Andrology 32(6):704-12.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Testicular testosterone levels
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD13-GD21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	676281

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test material source (Sigma-Aldrich) and purity (99%) were reported. The study did not include the certificate of analysis (or catalogue number), and the test material was not verified by the performing laboratory. Certificates of analysis are generally available on the supplier's website. Animals were dosed via gavage in corn oil and the gavage volume (5mL/kg) was appropriate. No details on the preparation, storage, or stability of the test solutions were provided. Doses were reported in mg/kg-day. It is not specified whether doses were adjusted daily based on measured body weights. Doses were not analytically verified.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD13-21. This exposure covers the period of post-implantation embryonic development, and the critical windows of organogenesis and male sexual differentiation.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Testosterone levels were measured using an ELISA assay from presumably pooled samples from the right testes of 1-2 males (GD21) per litter, and 6-8 litters per dose. There are no major concerns about the sample size used. The study text noted that samples were measured in a single run, suggesting the lack of replicates. Only a single dose was tested. The dose selected was either not expected to suppress testicular testosterone levels, or only to produce small changes and was justified by the authors. The study was focused on other endpoints that were presumably more sensitive. There are no concerns about the test model used.
	Metric 9:	Results presentation	High	Results were reported in a figure (bar graph) showing means ± SEM. Statistical significance and sample size (number of litters and individual fetuses) were shown. Litters were used as the experimental unit. Individual animal data were not provided

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Rajagopal, G., Bhaskaran, R. S., Karundevi, B. (2019). Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat F1 male offspring. Journal of Applied Toxicology 39(5):751-763.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-F1 males: blood glucose, serum insulin, insulin resistance, body weight, serum AST, ALT, ALP; hepatic glycogen concentration; enzymatic activity (i.e., glycogen synthase; glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); protein expression (i.e., Beta-arrestin; c-Src; phosphorylated/non-phosphorylated IR-beta, IRS-1, AKT, FoxO1, GSK3beta); mRNA levels (i.e., glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); transcription factor FoxO1 interaction with gene promoters glucose-6-phosphatase and phosphoenolpyruvate carboxykinase; serum urea, creatinine, testosterone and estradiol.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)-F0- lactation (PND 1-21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	5507636

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 identified as di-(2-ethylhexyl) phthalate (DEHP). The vendor source was reported; the purity was not specifically stated, but since the substance was analytical grade, a purity of ≥99.50 was obtained from the product specifications reported by the vendor. Test model (species, strain, parity, and age of the dams) and animal husbandry (cage type, light/dark cycle schedule, and food and water availability) details were reported. The experimental design including the number of animals per group, endpoint evaluation methods, and quantitative results for all outcomes were reported. No specific testing guideline or indication of GLP was reported. Missing animal husbandry information included temperature and humidity, and animal source was not reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	No information on the methods of allocation or randomization of animals into test groups, or for the selection of males used to assess specific endpoints was provided.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, the endpoints evaluated were not subjective in nature.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	The study included an appropriate vehicle control group, and the control responses were appropriate. No positive control was used. No body weight or food consumption data were reported for the dams. General animal husbandry conditions and procedures that were reported were limited to cage type, light/dark cycle schedule, and food and water availability, and these were consistent across groups. It was not reported whether measures were taken to minimize exposure to other plasticizers in food, bedding, or water dispensing and caging materials. The test substance is a known endocrine disrupter.
Domain 4: Selective Reporting and Attrition				

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Study Citation: Rajagopal, G., Bhaskaran, R. S., Karundevi, B. (2019). Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat F1 male offspring. Journal of Applied Toxicology 39(5):751-763.

Health Outcome(s) and Reported Health Effect(s): Reproductive/Developmental-F1 males: blood glucose, serum insulin, insulin resistance, body weight, serum AST, ALT, ALP; hepatic glycogen concentration; enzymatic activity (i.e., glycogen synthase; glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); protein expression (i.e., Beta-arrestin; c-Src; phosphorylated/non-phosphorylated IR-beta, IRS-1, AKT, FoxO1, GSK3beta); mRNA levels (i.e., glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); transcription factor FoxO1 interaction with gene promoters glucose-6-phosphatase and phosphoenolpyruvate carboxykinase; serum urea, creatinine, testosterone and estradiol.

Duration and Exposure Route: Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)-F0- lactation (PND 1-21)

Species: Rat-Wistar - [rat]-Female

Chemical: Diethylhexyl Phthalate- Parent compound

HERO ID: 5507636

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Results were reported for all outcomes and sample sizes were clearly described and included in the results. Insufficient information was provided to determine animal attrition. No mortality results or details of animal health were provided. Since the sample sizes were small (6 out of 36 males/group were used for most endpoints), it cannot be determined whether any animals died.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were exposed to DEHP via oral gavage. The vendor source was reported; the purity was not specifically stated, but since the substance was analytical grade, a purity of ≥99.50 was obtained from the product specifications reported by the vendor. The lot number, preparation details, and storage conditions of the test solutions were not reported. The route and method of exposure were suited to the test substance. Doses in mg/kg-day were provided; there is no indication that the test solutions were analytically verified. The gavage volume was not reported.
	Metric 7:	Exposure timing, frequency, and duration	High	The gavage dosing frequency (daily) and duration are considered adequate. The exposure timing of the dams from gestational day 9 to postnatal day 21 (lactation period) does not adhere to a specific reproductive/developmental testing guideline (e.g., OECD 414, 421), but this study was specifically interested in studying the effects of gestational and lactational exposure on insulin signalling and glucoregulatory effects.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	Only two exposure groups were tested but spacing was adequate to observe a concentration-related response. However, the authors did not justify the selected doses and the spacing was not adequate to identify a NOAEL (effects observed in all treatment groups). The source of the animals was not reported. Only 6 dams per group were tested, and only male F1 offspring were evaluated without justification. For most endpoints, only 1 male per dam was tested (n = 6/group); therefore, the study was not designed to assess litter effects. The sample sizes were sufficient to conduct statistical analysis. No testing guideline was followed. The study had a narrow focus and therefore did not include an assessment of any maternal endpoints including food intake, or, clinical signs, organ weights, macroscopic evaluations or histology of the target organs/systems in either dams or offspring. F1 males were appropriately fasted prior to nutritional/metabolic assessments (i.e., blood glucose, serum insulin, insulin resistance). The methods of the outcome assessment were clearly described and were consistent across groups.

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Study Citation:	Rajagopal, G., Bhaskaran, R. S., Karundevi, B. (2019). Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat F1 male offspring. Journal of Applied Toxicology 39(5):751-763.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-F1 males: blood glucose, serum insulin, insulin resistance, body weight, serum AST, ALT, ALP; hepatic glycogen concentration; enzymatic activity (i.e., glycogen synthase; glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); protein expression (i.e., Beta-arrestin; c-Src; phosphorylated/non-phosphorylated IR-beta, IRS-1, AKT, FoxO1, GSK3beta); mRNA levels (i.e., glucose-6-phosphatase; phosphoenolpyruvate carboxykinase); transcription factor FoxO1 interaction with gene promoters glucose-6-phosphatase and phosphoenolpyruvate carboxykinase; serum urea, creatinine, testosterone and estradiol.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)-F0- lactation (PND 1-21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	5507636

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Results were adequately reported in the text and quantitatively. Data were presented as means ± standard error and sample sizes were included in each figure. Western blot figures of the protein bands were not labeled to indicate the specific dose group. Statistical significance is shown and statistical methods were described and appropriate. Individual animal data were not provided. It would have been appropriate to assess some endpoints (e.g., birth weights) using the litter as the statistical unit; however, litters were culled, presumably immediately, to 6 males per litter, and only 1 male per litter was used for any given endpoint.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Rajesh, P., Balasubramanian, K. (2014). Phthalate exposure in utero causes epigenetic changes and impairs insulin signalling. Journal of Endocrinology 223(1):47-66.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Apical endpoints: Lean body weight, fat weight, fasting blood glucose and insulin levels. Mechanistic endpoints: gene expression, epigenetic modification (DNA methylation, ChIP), protein levels (Western, immunohistochemistry) of molecules involved in insulin signalling and glucose regulation.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2519077

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

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Study Citation:	Rajesh, P., Balasubramanian, K. (2014). Phthalate exposure in utero causes epigenetic changes and impairs insulin signalling. Journal of Endocrinology 223(1):47-66.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Apical endpoints: Lean body weight, fat weight, fasting blood glucose and insulin levels. Mechanistic endpoints: gene expression, epigenetic modification (DNA methylation, ChIP), protein levels (Western, immunohistochemistry) of molecules involved in insulin signalling and glucose regulation.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2519077

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Mortality, pup survival, or survival of offspring until the end of the study were not measured endpoints. For one outcome (glucose and insulin tolerance tests), it specifies that the tests were conducted on 6 male and 6 female offspring from different litters with only 1 offspring/sex/litter); since there were 6 pregnant females exposed per group, this suggests that at least all of the treated females survived and that six litters were generated. Results for all pre-specified outcomes were reported, and there was no indication of selective reporting.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The exact chemical source cannot be determined; however, all of the listed sources are appropriate. There was no independent analytical verification of the test substance or the purity, and the doses were not analytically verified. No information on the preparation of the test solutions, confirmation of homogeneity, or stability was provided. The exposure route (gavage) was appropriate for the test substance. The gavage volume was 2.0 mL/kg bw and the dosage was adjusted daily for maternal body weight changes. The noted uncertainties are expected to have a minimal impact on the interpretation of the results.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration (daily from GD 9-21 or parturition) were reported and were appropriate for the purposes of the study, although beyond indicating the desire to expose during gestation, the authors did not justify the selected window. All animals were dosed at the same time of day.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The study used Wistar rats. The use of the specific strain was not justified, but the animals appeared to be appropriate. This was not a guideline study, so there were no rules for the number of animals used. The study used 6 pregnant females/dose and culled each litter to 4/sex on PND1. All of the protocols were described in sufficient detail and were sensitive to the outcomes of interest. The study used three dose groups, plus a control, allowing for the data to show a dose response. The doses/spacing did not allow for the determination of a NOAEL (significant effects were observed in all dose groups), but this was not a goal of the study. The sample sizes were sufficient to allow for statistical analysis, and animals from each litter were represented. The outcomes were consistently assessed across groups.
	Metric 9:	Results presentation	High	Data were reported by sex for all dose groups, as means ± SE; the sample size (n) and statistical significance were noted. Statistical methods were clearly described and appropriate for the datasets.

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Apical endpoints: Lean body weight, fat weight, fasting blood glucose and insulin levels. Mechanistic endpoints: gene expression, epigenetic modification (DNA methylation, ChIP), protein levels (Western, immunohistochemistry) of molecules involved in insulin signalling and glucose regulation.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 9-21)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2519077

Domain	Metric	Rating	Comments

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Saillenfait, A. M., Sabaté, J. P., Robert, A., Rouiller-Fabre, V., Roudot, A. C., Moison, D., Denis, F. (2013). Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. Journal of Applied Toxicology 33(9):1027-1035.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Fetal ex vivo testosterone production.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-19)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000935

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical and important information was reported. Reported information included information on the test substance (name and source, purity, CASRN), the test model (species, strain, sex, source, initial body weights, and animal parity), animal husbandry details (food and water availability, temperature, humidity, and light cycle), number per cage, exposure methods, experimental design, endpoint evaluations, and presentation of results. Starting age was not reported, however as the animal life stage is implied by the animal's parity, this deficiency does not have a negative impact on the quality of the study.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Dams were randomly assigned to treatment groups by stratified randomization. The authors reported that mean body weights at GD0 did not differ among treatment groups. It was not specified how dams or litters were selected for the various outcomes assessed, or how fetuses were selected for measurement of testosterone production.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not specified but the outcome was measured using a standard laboratory kit.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Negative vehicle (olive oil) controls were included and gave the expected results. The study did not report taking measures to minimise the exposure to other plasticizers. Animals were housed in polycarbonate cages. Food, tap water, and bedding were not tested for contaminates, and the materials used to dispense water to the animals were not specified. The presence of contaminants could impact the study results; however, housing and husbandry conditions appear to be consistent across groups. Consistency of other potentially confounding factors (e.g., body weight, food or water intake) was not reported.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Saillenfait, A. M., Sabaté, J. P., Robert, A., Rouiller-Fabre, V., Roudot, A. C., Moison, D., Denis, F. (2013). Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. Journal of Applied Toxicology 33(9):1027-1035.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Fetal ex vivo testosterone production.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-19)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000935

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Insufficient information was provided to evaluate attrition or selective reporting. The total number of dams initially included in each test group was not specified. The methods indicated the endpoint was assessed using both testes from 3 male fetuses from 8-12 litters/dose group. The figure showing results specifies that the data were from 16 controls and 8 litters per treatment group. It is unclear whether any attrition occurred in any other litters.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test material source and purity (>98%) were reported and the identity and purity of the test substance were confirmed using GC/MS. The test substance stability and storage were reported. Details of the preparation of the test solutions were not provided. An olive oil vehicle was used. Animals were dosed via gavage, the gavage volume (5 mL/kg) was appropriate. Doses were adjusted every three days based on dam body weights (daily would be preferred). Analytical measurements were not made.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed from GD 12-19. This exposure covers the critical period of male reproductive tract differentiation in rats.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	There are no major concerns regarding the specificity and validity of the protocol for measuring testosterone production. Testosterone production was measured individually ex vivo from both testes from 3 fetuses/litter (6 samples per litter), and tests were conducted on 8-12 litters per group (data reported for 16 control litters and 8 litters per treatment group). The assay used a 3-hr incubation period and testosterone levels were measured using TFC-MS/MS. The limit of quantitation was specified. The study tested two doses, using DEHP as a positive control for dose-response assays on a different phthalate. Since the authors justified their dose group spacing by using DEHP as a positive control, effects were observed at both doses of DEHP examined and a NOAEL for this endpoint cannot be determined. The test species and strain were appropriate for the study type.
	Metric 9:	Results presentation	High	Results were reported in a figure (bar graph) showing means ± SD. Statistical significance and sample sizes were noted. The litter was used as the experimental unit. Individual animal data were not provided.

Additional Comments: Only ex-vivo testicular testosterone was evaluated for data quality.

Overall Quality Determination — High

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Study Citation:	Saillenfait, A. M., Sabaté, J. P., Robert, A., Rouiller-Fabre, V., Roudot, A. C., Moison, D., Denis, F. (2013). Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. Journal of Applied Toxicology 33(9):1027-1035.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-Fetal ex vivo testosterone production.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD12-19)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2000935

Domain	Metric	Rating	Comments

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

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

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

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes. Table footnotes did not specify the number of animals included in each group for analysis, it is unclear if all animals were evaluated and included in analysis.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Purity was not independently verified by the performing laboratory. The basal diet was mixed with 0.01, 0.03, and 0.09% of DEHP and formed into pellets. No additional details were provided on preparation or storage of the test material. Target test concentrations in food (%) were reported; there is no indication that analysis was done. The authors calculated dose equivalents in mg/kg/day for all groups; however, it was not specified how these calculations were made.
	Metric 7:	Exposure timing, frequency, and duration	High	F0 animals were exposed for 4 weeks prior to mating, during mating and through lactation. This agrees with OECD 422 guidelines. Exposure was consistent across study groups. Groups were treated concurrently. Dose rationale was based on several other reproductive and developmental studies previously performed using DEHP.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The animal species was appropriate. The number of animals/group was appropriate as group sizes were large enough to yield at least 8 pregnant females/group per OECD 422 guidance. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups and dose spacing was adequate. Outcome methodologies for the F0 and F1 generations were adequately reported and sensitive for the endpoints assessed.
	Metric 9:	Results presentation	Low	Results for some reproductive/developmental endpoints were shown in tables (shown as means $\pm$ SD), however other endpoints were reported as negative in text (data not shown). Statistical analysis methods were reported and statistical significance was noted in tables. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided. As noted by Dishaw et al., 2020, the presentation of offspring data as means of individual animals, rather than as litter means, has the potential to overestimate the statistical significance of experimental findings.

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Medium**	

Study Citation:	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	732820

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name (bis(2- ethylhexyl) phthalate (DEHP), a CASRN was not provided). The source and purity (>97%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins (4 weeks old at purchase). Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, number of animals/cage) were reported. Cage type and bedding type were reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as % in food and estimated based on food take. The duration was reported. Target, but not analytical concentrations in the food were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	Allocation methods were not reported for F0 animals.
	Metric 3:	Observational Bias / Blinding Changes	Medium	BW and food intake: Blinding was not reported however endpoints were not subjective in nature (body weight and food intake). Neuro: Blinding was not reported however exploratory behavior was assessed in a non-subjective manner using detectors of near-infrared photosensors for measuring spontaneous motor activity.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Average food intake was reported for all periods (premating, mating, gestation, lactation and F1 generation) and was similar between the groups; suggesting palatability was not an issue. Body weights of maternal animals were not shown, but were reported to be similar to controls during preconception and mating and "the average body weight of dams showed no significant adverse effects during the gestation and lactation periods". A negative control group was included (basal diet) and responses were appropriate. Housing conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes. Table footnotes did not specify the number of animals included in each group for analysis, it is unclear if all animals were evaluated and included in analysis.

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Study Citation:	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	732820

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Purity was not independently verified by the performing laboratory. The basal diet was mixed with 0.01, 0.03, and 0.09% of DEHP and formed into pellets. No additional details were provided on preparation or storage of the test material. Target test concentrations in food (%) were reported; there is no indication that analysis was done. The authors calculated dose equivalents in mg/kg/day for all groups; however, it was not specified how these calculations were made.
	Metric 7:	Exposure timing, frequency, and duration	High	F0 animals were exposed for 4 weeks prior to mating, during mating and through lactation. This agrees with OECD 422 guidelines. Exposure was consistent across study groups. Groups were treated concurrently. Dose rationale was based on several other reproductive and developmental studies previously performed using DEHP.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The animal species was appropriate. The number of animals/group was appropriate as group sizes were large enough to yield at least 8 pregnant females/group per OECD 422 guidance. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups and dose spacing was adequate. Outcome methodologies were adequately reported and sensitive for the endpoints assessed.
	Metric 9:	Results presentation	Medium	Food intake was reported in Table as mean intake +/- SD. Body weights and exploratory behavior effects were reported as negative in text (data not shown). Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1- post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	732820

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The test substance was identified by name (bis(2- ethylhexyl) phthalate (DEHP), a CASRN was not provided). The source and purity (>97%) were reported. Test animal species, strain, sex, age, and source were reported. It was not specified if mice were virgins (4 weeks old at purchase). Initial body weights were not reported. Husbandry conditions (temperature, humidity, light cycle, number of animals/cage) were reported. Cage type and bedding type were reported. Food and water were available ad libitum. Route of exposure was reported. Dose levels were reported as % in food and estimated based on food take. The duration was reported. Target, but not analytical concentrations in the food were reported. Endpoint evaluation methods were reported along with quantitative data.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	Allocation methods were not reported for F0 animals.
	Metric 3:	Observational Bias / Blinding Changes	Medium	BW and food intake: Blinding was not reported however endpoints were not subjective in nature (body weight and food intake). Neuro: Blinding was not reported however exploratory behavior was assessed in a non-subjective manner using detectors of near-infrared photosensors for measuring spontaneous motor activity.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	Average food intake was reported for all periods (premating, mating, gestation, lactation and F1 generation) and was similar between the groups; suggesting palatability was not an issue. Body weights of maternal animals were not shown, but were reported to be similar to controls during preconception and mating and "the average body weight of dams showed no significant adverse effects during the gestation and lactation periods". A negative control group was included (basal diet) and responses were appropriate. Housing conditions were consistent across groups. The study report did not indicate whether approaches were used to reduce exposure of test animals to plasticizers. There was also no indication of whether test animal bedding, food, and water were analyzed for the presence of contaminants, such as phthalates, which might impact the results and validity of the study. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Data were reported for all outcomes. Table footnotes did not specify the number of animals included in each group for analysis, it is unclear if all animals were evaluated and included in analysis.

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Study Citation:	Tanaka, T. (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food and Chemical Toxicology 40(10):1499-1506.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic-Body weight and food intake-Neurological/Behavioral-Exploratory behavior
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-1-F0- premating (4 weeks)-F0- mating (5 days)-F0 - gestation (14 days)-F0- lactation (4 weeks)-F1-post-natal-F0- premating (4 weeks)-F0- mating (5 days)-F1- post-natal
Species:	Mouse-CD-1 - [mouse]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	732820

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The source and purity of the test substance was reported. Purity was not independently verified by the performing laboratory. The basal diet was mixed with 0.01, 0.03, and 0.09% of DEHP and formed into pellets. No additional details were provided on preparation or storage of the test material. Target test concentrations in food (%) were reported; there is no indication that analysis was done. The authors calculated dose equivalents in mg/kg/day for all groups; however, it was not specified how these calculations were made.
	Metric 7:	Exposure timing, frequency, and duration	High	F0 animals were exposed for 4 weeks prior to mating, during mating and through lactation. This agrees with OECD 422 guidelines. Exposure was consistent across study groups. Groups were treated concurrently. Dose rationale was based on several other reproductive and developmental studies previously performed using DEHP.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	No guideline was specified. The animal species was appropriate. The number of animals/group was appropriate as group sizes were large enough to yield at least 8 pregnant females/group per OECD 422 guidance. Sample sizes were sufficient to allow for statistical analysis. The number of dose groups and dose spacing was adequate. Outcome methodologies were adequately reported and sensitive for the endpoints assessed.
	Metric 9:	Results presentation	Medium	Food intake was reported in Table as mean intake +/- SD. Body weights and exploratory behavior effects were reported as negative in text (data not shown). Statistical analysis methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

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

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

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. The number of exposure groups and spacing for this range-finding study were based on information from other studies on this chemical of interest. The endpoints evaluated were appropriate for a range-finding study. Sample sizes were specified and were adequate. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 8 mating pairs.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — High

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

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

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. The number of exposure groups and spacing for this range-finding study were based on information from other studies on this chemical of interest. The endpoints evaluated were appropriate for a range-finding study. Sample sizes were specified and were adequate. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 8 mating pairs.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — High

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

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

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The methods of outcome assessment were adequately described. The methods were sensitive to the outcomes of interest and endpoint evaluations included all groups. The number of exposure groups and spacing for this range-finding study were based on information from other studies on this chemical of interest. The endpoints evaluated were appropriate for a range-finding study. Sample sizes were specified and were adequate. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 8 mating pairs.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — High

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

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

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Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

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

Additional Comments: None

Overall Quality Determination — High

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Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

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

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Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

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

Additional Comments: None

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Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
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Domain	Metric		Rating	Comments
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Health Outcome(s) and Reported Health Effect(s):	Mortality-Mortality-Nutritional/Metabolic-Adult body weights, food and water consumption-Reproductive/Developmental-Reproductive and developmental parameters from: F0 (F1a, F1b, F1c), F1 (F2a, F2b, F2c), F2 (F3a, F3b, F3c) matings (litters), and from F1c and F2c crossover mating experiments. Endpoints include reproductive performance, standard litter parameters, growth and reproductive development of offspring (e.g., preputial separation, testis decent, vaginal opening), and reproductive tract malformations (RTMs). Reproductive organ weights of adults, estrous cyclicity, sperm parameters, gross observations.-Hepatic/Liver-Liver weights, gross necropsy, histopathology-Renal/Kidney-Kidney weights, gross necropsy, histopathology (including the bladder)-Other (please specify below) (Endocrine)-Adrenals and pituitary organ weights, gross necropsy, histopathology
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

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

Additional Comments: None

Overall Quality Determination — High

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

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

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

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

Additional Comments: None

Overall Quality Determination — High

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

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

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

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

Additional Comments: None

Overall Quality Determination — High

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as ”per Amendment 1.”
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination High

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

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as ”per Amendment 1.”
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination High

Study Citation:	TherImmune Research Corporation, (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet: Final report.
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Species:	Rat-Sprague-Dawley - [rat]-Both
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HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

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

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

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — High

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Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and important information was provided. The test material name, CASRN, structure, source, and purity were reported. The study used Crl:CD BR rats, the animal source, sex, age, and starting weights were provided. Parity was not specified. All animal husbandry details were reported including the number of animals per cage. Exposure details (route, methods, numbers of animals etc.,) protocols for outcome assessment and quantitative data were provided for most outcomes.
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Health Outcome(s) and Reported Health Effect(s):	Skin/Connective Tissue-Clinical signs: abrasions and alopecia, reddened areas (erythema)-Neurological/Behavioral-Clinical signs: Paralysis, hunched posture, languid behavior, lethargy, tremors, prostration; gross necropsy and histopathology of related tissues.-Ocular/Sensory-Clinical signs: Discharge from the eyes, lacrimation, squinting, bulging or protruding eyes, opacity of the eye-Other (please specify below) (General clinical signs)-Clinical signs:, urine stains, few feces, ulcers, swelling, small stationary tissue mass, paleness, anorexia, rough haircoat, thinness, hypothermia, dyspnea, missing anatomy, malocclusion, papilloma, obesity-Thyroid-Thyroid/parathyroid gross necropsy, histopathology if any gross findings-Gastrointestinal-Gross necropsy and histopathology if any gross findings in related tissues-Immune/Hematological-Gross necropsy and histopathology if any gross findings in related tissues-Lung/Respiratory-Gross necropsy and histopathology if any gross findings in related tissues-Cardiovascular-Gross necropsy and histopathology if any gross findings in related tissues
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-3-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F0 - gestation (~21 days)-F0- lactation (21 days)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1 - gestation (~21 days)-F1- lactation (21 days)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2 - gestation (~21 days)-F2- lactation (21 days)-F2- post-natal (weaning through necropsy)-F0- premating (6 weeks)-F0- mating (9 weeks cohabitation)-F1- premating (~60 days (PND21 to PND81))-F1- mating (9 weeks cohabitation)-F1- post-natal (weaning through necropsy)-F2- premating (~60 days (PND21 to PND81))-F2- mating (9 weeks cohabitation)-F2- post-natal (weaning through necropsy)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as ”per Amendment 1.”
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — High

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

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Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Chemical:	Diethylhexyl Phthalate- Parent compound
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Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination High

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Domain	Metric	Rating	Comments
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Additional Comments: None

Overall Quality Determination — High

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

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

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Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as "per Amendment 1."
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	3108900 Linked HERO ID(s): 3108900, 1334515, 5556685

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination High

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

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Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	High	Some deaths were reported, but the incidences were low, occurred in all groups, and had no impact on the study results. Data for all of the outcomes were provided; there is no indication of selective reporting although, some animals originally allocated for necropsy were not necropsied as ”per Amendment 1.”
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	High	The test chemical source and purity (>99%) were reported. The test material was provided with a certificate of analysis and an independent evaluation was performed. Food was mixed to homogeneity. Preparation and storage details were reported, and all dose formulations were analyzed using HPLC. Measured concentrations were within 92.5 to 128.3% of nominal. No concerns regarding the method of administration (diet) were identified, and the route was appropriate for the study type.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were appropriate for a continuous breeding study and the outcomes of interest including additional assessments of development as groups of animals age.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	The methods of outcome assessment were adequately described, and animals from all treatment groups were sampled. This study did not include comprehensive histopathological analysis on all tissues, and only measured select organ weights. Data for the target organs/tissues noted were primarily limited to clinical observations or gross necropsy findings, and these endpoints alone are not considered to be sensitive for the identification of organ-specific toxicity. Histopathology was only conducted if gross findings were observed. Dose concentrations and spacing were based on a range-finding study and data from other publications. A high dose of 10,000 ppm was added ~2 weeks after the start of the main study but included its own control. Generally, for reproduction studies, enough mating pairs to generate 20 litters is preferred. This study only used 17 mating pairs. Sample sizes were clearly stated and were sufficient for statistical analysis.

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

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — High

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

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

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

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	Animals were observed for clinical signs, and low incidences of clinical signs relevant to the skin, neurological, and other (general) outcomes were reported. No further evaluation of these target organs/systems was conducted (e.g., histopathology) and clinical signs are not considered to be a sensitive measure of target organ toxicity. There are no concerns regarding sampling, and all dose groups were evaluated.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — Medium

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

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

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

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	Animals were observed for clinical signs, and low incidences of clinical signs relevant to the skin, neurological, and other (general) outcomes were reported. No further evaluation of these target organs/systems was conducted (e.g., histopathology) and clinical signs are not considered to be a sensitive measure of target organ toxicity. There are no concerns regarding sampling, and all dose groups were evaluated.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — Medium

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

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

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

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	Medium	In this extended dose range-finding study, animals were dosed continuously in their diets starting 1 week prior to mating, through mating (co-housed for 28 days), the gestational and lactation periods, and were sacrificed on PND21. The exact duration (total number of days) was not clearly specified, and generally, it is preferred that dosing start at least two weeks prior to mating. The deviations may be appropriate for a range-finding study. was sensitive for the outcomes of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	Animals were observed for clinical signs, and low incidences of clinical signs relevant to the skin, neurological, and other (general) outcomes were reported. No further evaluation of these target organs/systems was conducted (e.g., histopathology) and clinical signs are not considered to be a sensitive measure of target organ toxicity. There are no concerns regarding sampling, and all dose groups were evaluated.
	Metric 9:	Results presentation	High	Statistical methods were clearly described and the litter was used as the experimental unit where appropriate. All data were quantitatively reported as incidences or means ± SE, and individual animal data were provided.

Additional Comments: None

Overall Quality Determination — Medium

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

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

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

Domain	Metric		Rating	Comments
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	Quantitative results were reported for most, but not all outcomes described in the methods. It was stated that the number of male fetuses on GD 21 was recorded, however, no results were provided for this metric. For gene expression results from the cDNA microarray, only results for the animals receiving 100 or 500 mg/kg bw/day are provided. The absence of the 10 mg/kg bw/day group is not explained in the text. In addition, the authors reference Table 4-d in the text, however, there is no Table 4-d included in the paper. This suggests that some data may be missing from the paper. The authors stated that offspring body weights and the male-female ratio per litter were recorded on PND 1, however, no results were provided for these metrics. In addition, it was stated that offspring were weighed weekly between PND 1 and 63, however, these measured weights are not reported. Overall, these omissions are expected to significantly impact the interpretation of the results. Sample sizes were not clearly reported; there is insufficient information to determine whether any animal attrition occurred.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Pure Chemical Industries), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed; however, a certificate of analysis was likely available from the supplier. The authors reported doses in mg/kg bw/day; test substance concentrations in the test solutions were not analytically measured. It is also unclear whether doses were adjusted for daily body weight changes. Storage conditions were not reported. The gavage volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage volumes of the treatment groups were the same. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	Medium	For this study, the route, frequency, and duration of exposure (dams exposed orally via gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of GD 11 to 21 and did not include the period of organogenesis.
Domain 6: Outcome Measures and Results Display				

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

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

Additional Comments: None

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

Domain	Metric	Rating	Comments
Overall Quality Determination		**Low**	

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information were reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, the endpoints are simple measures (body weights). Blinding was not reported for clinical signs.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to significantly impact these endpoints (maternal body weights and clinical signs).
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors. This lack of data provided for these endpoints resulted in an uninformative designation.
Domain 5: Exposure Methods Sensitivity				

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Pure Chemical Industries), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed; however, a certificate of analysis was likely available from the supplier. The authors reported doses in mg/kg bw/day; test substance concentrations in the test solutions were not analytically measured. It is also unclear whether doses were adjusted for daily body weight changes. Storage conditions were not reported. The gavage volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage volumes of the treatment groups were the same. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (dams exposed orally via gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of GD 11 to 21.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an in-utero developmental toxicity study. The study does not state whether clinical signs were cage-side or detailed clinical observations with handling. The frequency of observations was not specified. The authors also do not give any information on the "abnormal behavior" observations. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose selection. The sample size (8 dams/group) was small but should have been sufficient for the endpoints of interest.
	Metric 9:	Results presentation	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors.

Additional Comments: None

Overall Quality Determination — Uninformative

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information were reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, the endpoints are simple measures (body weights). Blinding was not reported for clinical signs.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to significantly impact these endpoints (maternal body weights and clinical signs).
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors. This lack of data provided for these endpoints resulted in an uninformative designation.
Domain 5: Exposure Methods Sensitivity				

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Pure Chemical Industries), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed; however, a certificate of analysis was likely available from the supplier. The authors reported doses in mg/kg bw/day; test substance concentrations in the test solutions were not analytically measured. It is also unclear whether doses were adjusted for daily body weight changes. Storage conditions were not reported. The gavage volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage volumes of the treatment groups were the same. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (dams exposed orally via gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of GD 11 to 21.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an in-utero developmental toxicity study. The study does not state whether clinical signs were cage-side or detailed clinical observations with handling. The frequency of observations was not specified. The authors also do not give any information on the ”abnormal behavior” observations. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose selection. The sample size (8 dams/group) was small but should have been sufficient for the endpoints of interest.
	Metric 9:	Results presentation	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors.

Additional Comments: None

Overall Quality Determination — Uninformative

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical and some important information were reported in this study. The study included identification of the test substance (di-(2 ethylhexyl) phthalate), and source (Wako Pure Chemical Industries); test animal characteristics (species, strain, age, sex); general animal husbandry conditions (light/dark cycle, diet, water availability); exposure methods (method of administration); experimental design (frequency of exposure, number of animals per study group); and endpoint evaluation methods (quantitative and qualitative). The study lacked some important information including test animal characteristics (starting body weight), general animal husbandry conditions (temperature, humidity, and number of animals per cage), and exposure methods (purity of test substance). All critical information is reported, however, the missing important information is expected to substantially impact the interpretation of the results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The study did not report how the animals were allocated to study groups. No other methods to control for modifying factors across groups were noted.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported; however, the endpoints are simple measures (body weights). Blinding was not reported for clinical signs.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a negative control group, which received the vehicle (corn oil) by gavage. A positive control group receiving testosterone propionate, an androgen agonist, by gavage, was used as an indicator of androgenic activity. Animal husbandry conditions appeared to be consistent across study groups. However, there was no indication of whether test animal bedding or food was analyzed for the presence of contaminants, such as phthalates. Polycarbonate cages were used instead of wire cages. Food and water dispensing containers were not described. This is not expected to significantly impact these endpoints (maternal body weights and clinical signs).
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors. This lack of data provided for these endpoints resulted in an uninformative designation.
Domain 5: Exposure Methods Sensitivity				

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

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	In this study, test animals were gavaged with DEHP. The source of the test substance was reported (Wako Pure Chemical Industries), however, the purity of the test substance was not provided. In addition, independent analytical verification of the test substance was not performed; however, a certificate of analysis was likely available from the supplier. The authors reported doses in mg/kg bw/day; test substance concentrations in the test solutions were not analytically measured. It is also unclear whether doses were adjusted for daily body weight changes. Storage conditions were not reported. The gavage volume for the vehicle control group was 5 mL/kg. It was not stated whether the gavage volumes of the treatment groups were the same. There were very few details provided on test substance preparation for gavage. These uncertainties in the exposure characterization are expected to substantially impact the results.
	Metric 7:	Exposure timing, frequency, and duration	High	For this study, the route, frequency, and duration of exposure (dams exposed orally via gavage daily from GD 11 to 21) were appropriate for the endpoints of interest. However, the study authors did not provide an explanation for why they selected an exposure period of GD 11 to 21.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Low	This was an in-utero developmental toxicity study. The study does not state whether clinical signs were cage-side or detailed clinical observations with handling. The frequency of observations was not specified. The authors also do not give any information on the "abnormal behavior" observations. The number of exposure groups (0, 10, 100, and 500 mg/kg bw/day) was appropriate, however, there was no justification for the dose selection. The sample size (8 dams/group) was small but should have been sufficient for the endpoints of interest.
	Metric 9:	Results presentation	Uninformative	No results were provided for maternal body weights, observed clinical signs, or abnormal behaviors.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-F0-treated dams (serum estradiol); F1 (oocyte meiosis and folliculogenesis, gene expression, DNA Methylation, protein levels of Stra8); F2 (folliculogenesis)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (0.5-18.5 Days postcoitum)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2519060

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Low	All critical information was reported including the name of the test material (DEHP), the test species (mouse), the doses 0 and 40 ug/kg-day), duration of exposure (GD 0.5 to 18.5), and route (gavage), and quantitative results for at least one endpoint of information. Important information reported included the test species strain (CD-1), source (Vital River, Beijing, China), sex (females), animal husbandry conditions (temperature and light conditions), and outcome/endpoint evaluation methods. The study did not report test substance purity, animal age, starting body weights, parity, humidity, diet and water availability, number of per group, or number of animals per cage. The missing information is expected to significantly reduce the ability to evaluate the study.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The method of allocation of animals into study groups, or downstream selection of animals for various outcome assessments was not specified. There is no indication that randomization or other methods to control for important modifying factors across groups was used.
	Metric 3:	Observational Bias / Blinding Changes	Low	The study did not report the use of blinding, but most of the outcomes of interest were not subjective in nature. One outcome, the assignment of follicular stages was likely subjective in nature, and blinding would have been appropriate.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A concurrent negative vehicle control group was administered saline containing DMSO at a final concentration of 0.1%. The negative control responses appeared to be appropriate. The study did not evaluate standard potential variables of concern (e.g., food and water intake, body weights), and only minimal animal husbandry conditions were provided. DEHP is also a known endocrine disruptor and the outcomes of interest in this study were looking at effects related to endocrine disruption. It was not specified whether co-exposure to plasticizers was specifically avoided in this study or was at least consistent across groups. Positive controls were not included but were not necessary for this study.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-F0-treated dams (serum estradiol); F1 (oocyte meiosis and folliculogenesis, gene expression, DNA Methylation, protein levels of Stra8); F2 (folliculogenesis)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (0.5-18.5 Days postcoitum)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2519060

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	Selective reporting and attrition cannot be properly assessed because the study did not report the number of animals per group (one figure sampled 5 control and treated F0 dams, but it is not clear if this represents the number of animals per group). For most outcomes, it was only noted that most experiments were performed in triplicate (three independent trials), and some also included three replicates, but the actual sample sizes were also not specified. Data for all of the specified outcomes were reported.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The test substance was identified as diethylhexyl phthalate (DEHP) sourced from Sigma-Aldrich. The test material was not independently verified and the specific product (from Sigma) used in this study cannot be determined. The purity and/or grade were not reported and cannot be definitively verified on the supplier's website. The doses were not analytically verified. The method of exposure is assumed to be gavage; the study states that "water containing DEHP was given orally." In another study by the same group that appears to be on the same group of animals, it was indicated that the test substance was oraly delivered using an Eppendorf pipette with a disposable tip (Li et al., 2014; HERO 2519022). No gavage volume was specified and no details on the preparation (e.g., homogeneity, frequency), and stability of the test solutions were provided. The dose, 40 ug/kg-day was justified by the study authors.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure timing, frequency, and duration were based on results from previous studies. The exposure period (GD 0.5 to 18.5) covers the period of mammalian folliculogenesis was appropriate for the purpose of the study. The time of day of dosing was not specified. There is no indication that there were any inconsistencies across groups.
Domain 6: Outcome Measures and Results Display				

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Study Citation:	Zhang, X. F., Zhang, T., Han, Z., Liu, J. C., Liu, Y. P., Ma, J. Y., Li, L., Shen, W. (2014). Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reproduction, Fertility and Development 27(8):1213-1221.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental-F0-treated dams (serum estradiol); F1 (oocyte meiosis and folliculogenesis, gene expression, DNA Methylation, protein levels of Stra8); F2 (folliculogenesis)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (0.5-18.5 Days postcoitum)
Species:	Mouse-CD-1 - [mouse]-Female
Chemical:	Diethylhexyl Phthalate- Parent compound
HERO ID:	2519060

Domain	Metric		Rating	Comments
	Metric 8:	Endpoint sensitivity and specificity	Low	The purpose of the study was to investigate whether maternal exposure to DEHP affected fetal ovarian development. The endpoints (measurement of serum estradiol on GD 12.5, gene expression and bisulfate sequencing on germ cells at GD 13.5, determination of the meiotic status of oocytes on GD 17.5 in dames, and examination of ovaries from PND21 F1 and F2 offspring), were sensitive and appropriate for the purposes of the study. The details of the outcome assessment protocols were confusing and did not always clearly distinguish whether it was fetal or maternal samples being examined. The only figure reporting a sample size (n =5) was Fig. 2a, measuring estradiol levels in F0 pregnant dams. It was noted that some endpoints were assessed in triplicate. The outcomes were consistently assessed in the control and treatment groups. Only a single exposure group was tested, but the dose was justified by the study author, and the purpose of the study was not to identify a dose response. The test animal species/strain had been previously used by the same group and was obtained from a commercial source. It is unclear if the number of animals per group was appropriate because this information was not provided.
	Metric 9:	Results presentation	Low	Data for all endpoints were reported primarily as figures showing means ± SEM. Some figure legends specified that experiments were repeated at least three times, but did not specify how many animals were used to generate the data. Statistical significance was shown, and the methods of statistical analysis were adequately described.

Additional Comments: None

Overall Quality Determination Low