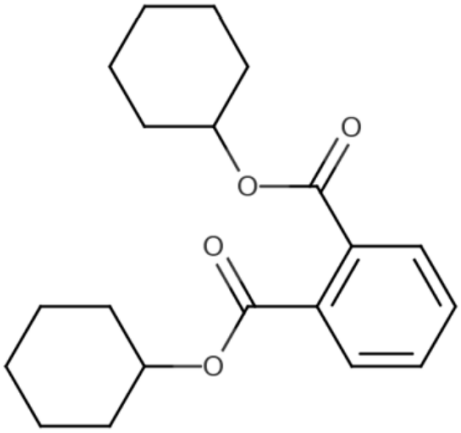
United States
Environmental Protection Agency

December 2025
Office of Chemical Safety and
Pollution Prevention

Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Dicyclohexyl Phthalate (DCHP) (1,2- Benzenedicarboxylic acid, 1,2-dicyclohexyl ester)

Systematic Review Support Document for the Risk Evaluation

CASRN: 84-61-7



December 2025

This supplemental file contains information regarding the data quality evaluation conducted for key references identified by EPA as described in the *Risk Evaluation for Dicyclohexyl Phthalate (DCHP) – Systematic Review Protocol*. EPA conducted data quality evaluation based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration into the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. For the data quality evaluation, EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as '2021 Draft Systematic Review Protocol'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Risk Evaluation for Dicyclohexyl Phthalate (DCHP) – Systematic Review Protocol*.

Table of Contents

Study Citation:	Ahbab, M. A., Barlas, N. (2015). Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalate on fetal testicular development in rats. Toxicology Letters 233(2):125-137.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Litters with resorptions, percentage resorption, offspring length (from head to tail), weight of male offspring, anogenital distance (AGD), GD20 fetal hormones including: testosterone, follicle stimulating hormone, inhibin B, Mullerian inhibiting substance/antimullarian hormone (MIS/AMH) and testes histopathology including: H and E staining, immunohistochemistry for 3-beta-hydroxysteroid dehydrogenase, and MIS/AMH, androgen receptor and proliferating cell nuclear antigen, determination of Leydig cell number and clusters.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	2914645

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical information is reported. Test animal source, species, strain, age, starting body weight were reported. Animal housing conditions such as temperature, humidity, light/dark cycle, food/water availability and number of animals per cage were reported. Test substance identity, purity, source and method of administration are reported. Frequency of exposure, number of animals per study group, life stage during exposure and endpoint evaluation methods are all reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly allocated into groups, but the authors did not describe the method of randomization.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No methods to reduce observational bias were described. The endpoints were generally objective in nature or based off of initial histopathology and were not subject to observational bias.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	An appropriate negative vehicle control was included and there was no response in the negative control. Potentially confounding variables such as maternal body weights and food/water consumption were measured and do not appear to be a concern.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All pre-prescribed outcomes are reported in the results and all animals are accounted for. There is no indication of animal attrition or any health outcomes unrelated to the exposure.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance source and purity are appropriate, but the authors did not perform an independent analytical verification of the test substance purity or dosing concentrations, which may have a minor impact on the results. The authors prepared the dosing solution fresh daily, so there are no concerns with test substance preparation. Test substance storage was not described in detail but is unlikely to be a concern. There are no concerns about the method of administration and the gavage volume is considered appropriate for dosing through corn oil vehicle according to OECD TG 414.

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Study Citation:	Ahbab, M. A., Barlas, N. (2015). Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalate on fetal testicular development in rats. Toxicology Letters 233(2):125-137.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Litters with resorptions, percentage resorption, offspring length (from head to tail), weight of male offspring, anogenital distance (AGD), GD20 fetal hormones including: testosterone, follicle stimulating hormone, inhibin B, Mullerian inhibiting substance/antimullarian hormone (MIS/AMH) and testes histopathology including: H and E staining, immunohistochemistry for 3-beta-hydroxysteroid dehydrogenase, and MIS/AMH, androgen receptor and proliferating cell nuclear antigen, determination of Leydig cell number and clusters.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	2914645

Domain	Metric		Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	The authors justified the exposure duration based on a window of sensitivity for male developmental/reproductive toxicity that was determined in previous publications. The duration and frequency of the exposure was sensitive to detect the endpoint of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The species is appropriate to detect the endpoint of interest. Sample size is lower than what is typically recommended for reproductive/developmental studies. Methods to assess the outcome were generally appropriate, and mechanistic outcomes are paired with more relevant apical endpoints. The timing of endpoint assessment was appropriate.
	Metric 9:	Results presentation	High	Statistical methods are appropriate and there is full quantitative presentation of the results, including measures of variance for continuous measures and incidence data for histopathological outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ahbab, M. A., Barlas, N. (2015). Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalate on fetal testicular development in rats. Toxicology Letters 233(2):125-137.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Maternal body weights, maternal food and water consumption.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	2914645

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	All critical information is reported. Test animal source, species, strain, age, starting body weight were reported. Animal housing conditions such as temperature, humidity, light/dark cycle, food/water availability and number of animals per cage were reported. Test substance identity, purity, source and method of administration are reported. Frequency of exposure, number of animals per study group, life stage during exposure and endpoint evaluation methods are all reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were randomly allocated into groups, but the authors did not describe the method of randomization.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No methods to reduce observational bias were described. The endpoints were objective in nature and not subject to observational bias.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	An appropriate negative vehicle control was included and there was no response in the negative control. Potentially confounding variables such as maternal body weights and food/water consumption were measured and do not appear to be a concern.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All pre-prescribed outcomes are reported in the results and all animals are accounted for. There is no indication of animal attrition or any health outcomes unrelated to the exposure.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Test substance source and purity are appropriate, but the authors did not perform an independent analytical verification of the test substance purity or dosing concentrations, which may have a minor impact on the results. The authors prepared the dosing solution fresh daily, so there are no concerns with test substance preparation. Test substance storage was not described in detail but is unlikely to be a concern. There are no concerns about the method of administration and the gavage volume is considered appropriate for dosing through corn oil vehicle according to OECD TG 414.
	Metric 7:	Exposure timing, frequency, and duration	High	The authors justified the exposure duration based on a window of sensitivity for male developmental/reproductive toxicity that was determined in previous publications. The duration and frequency of the exposure was sensitive to detect the endpoint of interest.

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Study Citation:	Ahbab, M. A., Barlas, N. (2015). Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalate on fetal testicular development in rats. Toxicology Letters 233(2):125-137.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Maternal body weights, maternal food and water consumption.
Duration and Exposure Route:	Oral-Gavage-Duration: Short-term (>1-30 days)-1-F0 - gestation (GD6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	2914645

Domain	Metric		Rating	Comments
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species is appropriate to detect the endpoint of interest. Sample size is appropriate to measure nutritional/metabolic effects. Methods to assess the outcome were generally appropriate, and mechanistic outcomes are paired with more relevant apical endpoints. The timing of endpoint assessment was appropriate.
	Metric 9:	Results presentation	High	Statistical methods are appropriate and there is full quantitative presentation of the results, including measures of variance for continuous measures.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ahbab, M. A., Barlas, N. (2013). Developmental effects of prenatal di-n-hexyl phthalate and dicyclohexyl phthalate exposure on reproductive tract of male rats: Postnatal outcomes. Food and Chemical Toxicology 51:123-136.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Sperm abnormalities, histopathology (testes, epididymides and prostate)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD 6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1639260

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information is reported. The test substance is identified by name and CASRN. Test substance source, purity and method of administration are reported. Test animal species, strain, sex, pregnancy status, source and animal housing conditions (including type of cage, number of animals per cage, light/dark cycle, temperature, humidity and food and water availability). Starting age, initial body weights, and parity are not reported. Endpoints and endpoint assessment methods are reported.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Animals were allocated into groups randomly, but the authors did not specify the method of randomization.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Methods to reduce observational bias were not described; however, endpoints were either quantitative (sperm count), evaluation of sperm morphology (microscopically), or initial histopathology.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	A negative vehicle control was included, and responses were appropriate. No positive control was included nor required for the study. Due to using gavage as the method of administration, there are no concerns for confounding from palatability. The authors do not report the age of the animals, so there could be unmeasured confounding from the age of pregnant dams, but this possibility cannot be confirmed nor ruled out. Body weights of maternal animals at initiation or during the study were not reported. There was no indication that experimental conditions, including husbandry, differed across the study groups. However, 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:	Ahbab, M. A., Barlas, N. (2013). Developmental effects of prenatal di-n-hexyl phthalate and dicyclohexyl phthalate exposure on reproductive tract of male rats: Postnatal outcomes. Food and Chemical Toxicology 51:123-136.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Sperm abnormalities, histopathology (testes, epididymides and prostate)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD 6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1639260

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Ten pregnant dams/group were treated from GD6-19 and allowed to deliver naturally. All pups remained with the dam for one month. Males were then separated and evaluated at PND 20 (n=10/group), PND 32 (n=8-10/group) or PND 90 (n=10/group). The study does not report any information on the pregnant dams (i.e. survival), how many litters were born, or the number of pups/litter. It is unclear if the 8-10 pups evaluated/time point/dose group all came from different litters or if some were siblings. No explanation is provided for why only 8 animals were examined on PND32 in the 20 mg/kg/day group (if there were not enough male pups born or if pups died). All prespecified outcomes appear in the results.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The reported purity, form and supplier of the test substance are appropriate (99% pure), but the authors do not perform an independent analytical verification of the test substance purity. Test substance storage was not described, but as DCHP is highly stable at room temperature, this deficiency is unlikely to present a large problem. The test substance was prepared fresh daily, and the nominal administered dose is presented in mg/kg/day units, though there is no analytical confirmation of administered dose levels.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, frequency and duration of the exposure was sensitive and cover the known window of sensitivity for male reproductive defects. These exposure details were also consistent between groups.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species and sample size are appropriate to evaluate outcomes of interest. 3 dose levels are included and are sufficient to determine a LOAEL, but an additional lower dose would be required to determine a NOAEL. There are no concerns with the timing or consistency of outcome assessment. Outcome assessment methods for reproductive tract histopathology are generally appropriate and consistent with what is recommended by OECD guideline TG 414. Evaluations were performed for animals of all dose levels.
	Metric 9:	Results presentation	Low	The presentation of the results is complete with quantitative mean %s for sperm quality with measures of variance, and incidence data and representative images for male reproductive tract histopathology. Statistical methods are described. There is no indication that the litter was used as the experimental unit. Individual animal data were not provided.

Additional Comments: Only sperm quality and histopathology for testes, epididymis and prostate were evaluated for data quality.

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Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Sperm abnormalities, histopathology (testes, epididymides and prostate)
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-F0 - gestation (GD 6-19)
Species:	Rat-Other (Wistar albino)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1639260

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

Study Citation:	Ahbab, M. A., Güven, C., Koçkaya, E. A., Barlas, N. (2017). Comparative developmental toxicity evaluation of di- n-hexyl phthalate and dicyclohexyl phthalate in rats. Toxicology and Industrial Health 33(9):696-716.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: skeletal retardation, delayed ossification in offspring, and placental histopathology.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-19)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	4729046

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical and most important information were reported. The test material was identified as dicyclohexyl phthalate DCHP (CAS no. 84-61-7) and source was reported. Other reported information included details on the test model (species, strain, source, and pregnancy); animal husbandry (food and water availability, temperature, humidity, light cycle, number per cage); exposure details, experimental design, number of animals per group, endpoint evaluation methods, and results for the endpoint of interest. Missing information included the test chemical purity, animal starting age and body weights.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	The rats were randomly allocated but the method of allocation was not further described.
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding was not reported, however, the endpoints evaluated were quantitative (bone biometrics and staining) or initial histopathological evaluation.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a concurrent vehicle control that gave expected response. No positive control was included nor required for the study. Study groups were maintained, exposed and evaluated under comparable conditions. There was no indication of confounding variables. The study did not report taking measures to minimize 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.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	There was no apparent attrition. Sampling for bone staining and biometrics were randomly selected from one per sex per litter for each group. Placental histopathology was evaluated from one placenta per litter for each group. Quantitative outcomes were reported for each group for each outcome.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Ahbab, M. A., Güven, C., Koçkaya, E. A., Barlas, N. (2017). Comparative developmental toxicity evaluation of di- n-hexyl phthalate and dicyclohexyl phthalate in rats. Toxicology and Industrial Health 33(9):696-716.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: skeletal retardation, delayed ossification in offspring, and placental histopathology.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-19)
Species:	Rat-Wistar - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	4729046

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The source of the test material (Aldrich Chemistry) was reported, but purity was not. There is no source purity nor independent laboratory analytical verification. Limited details of preparation and storage were provided; dissolved in corn oil fresh daily. Doses were not analytically verified. Animals were treated via gavage, and appropriate method, with a consistent volume of 0.25 mL in all groups. Doses were adjusted based on dam's body weight.
	Metric 7:	Exposure timing, frequency, and duration	High	Animal were dosed daily during gestation days 6-19 which is sensitive and covers the specific window of fetal skeletal development.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	No guideline was specified; however, the study was conducted in a similar manner to OECD 414 (with deviations) using pregnant Wistar rats. The number of animals per group (10) was less than recommended (20).Sample size (one per sex per litter or one per litter) was less than recommended by guideline but was randomized and justified. The doses and dose spacing were justified and adequate. The doses used 2, 100, and 500 mg/kg/day were selected so the high dose does not exceed the LD50, and the low dose is based on previously reported NOAEL. The methods used were reliable and sensitive for the outcomes of interest and consistently implemented. While some parameters were not measured (thyroid, soft tissue), additional outcomes were included (placental histopathology and immunohistochemistry, and bone biometrics), which is consistent with the specific goal of the study.
	Metric 9:	Results presentation	High	Results for skeletal retardation, delayed ossification, and placental histopathology were reported in figures and tables with means and variation (SEM) and included sample sizesStatistical analyses were reported, and normality and homogeneity tests were appropriate. Use of two sample t test was used to detect differences between groups and is adequate. Sufficient data are also provided for independent analysis.

Additional Comments: None

Overall Quality Determination Medium

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

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	Good. Important information is provided for test animals, exposure methods, experimental design, endpoint evaluations, and the presentation of results.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Medium	Adequate. Pregnant rats were randomly assigned to treatment groups on GD 14 in a manner that provided each group with similar means and variances in body weight. The method for randomization is not detailed, but this description indicates that normalization procedures were performed to balance important variables across groups.
	Metric 3:	Observational Bias / Blinding Changes	Medium	All outcomes: Adequate. The paper did not indicate that whether investigators were blinded during outcome assessment. However, via personal correspondence, authors indicated that fetal dissections were performed by investigators that were unaware of the treatment group. Potential concern for bias was mitigated because all outcomes reported in this study are relatively objective measurements.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	High	Good. Vehicle (laboratory grade corn oil) and gavage volume were the same in control and treatment groups. Additionally, water was tested monthly for Pseudomonas and every four months for a suite of chemicals, including pesticides and heavy metals. The experimental conditions described provided no indication of different practices across treatment groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	Adequate. All endpoints described in methods were reported qualitatively or quantitatively. Data are complete for all endpoints (generally 3-4 dams per group) except for T production data in Block 23, which is only shown for 2 animals. The authors do not provide an explanation.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	Adequate. The authors tested several ”blocks” of animals, and the source, purity, and lot # was reported for each block. Chemicals were supplied by Aldrich and was 99% pure in all cases, although it is not clear that the authors independently verified the chemical purity or stability. Dams were weighed and dosed daily with test chemical in laboratory grade corn oil.

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

Domain		Metric	Rating	Comments
	Metric 7:	Exposure timing, frequency, and duration	High	Testosterone: Good. Pregnant dams were dosed daily with test substance from GD 14-18, which coincides with the critical window of male sexual differentiation (Dent et al. 2015 [3452649]; Scott et al. 2009 [673313]).
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Testosterone: Good. No concerns regarding the specificity and validity of the protocols and measures were identified. Testosterone production in an ex vivo assay was measured using a commercial radioimmunoassay kit according to the manufacturer's protocols. One testis each was dissected from 3 male fetuses/litter; it is not clear whether the individual testes were left or right, so differential/bilateral effects are not evaluated. Sample size is small (n=3 dams/dose group), but was validated by the authors to have sufficient statistical power to evaluate changes in fetal testosterone production, although authors stated that changes less than 20-25% may not be consistently detected.
	Metric 9:	Results presentation	High	All outcomes: Good. There are no notable concerns about the way the results are analyzed or presented.

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

Overall Quality Determination High

Study Citation:	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:	Dicyclohexyl 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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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:	Dicyclohexyl 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. 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	DCHP is not listed in the chemical information tab in the supplemental file; however, 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 it 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: None

Overall Quality Determination High

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 (Harlan Sprague Dawley)-Female
Chemical:	Dicyclohexyl 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.
Domain 5: Exposure Methods Sensitivity				

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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 (Harlan Sprague Dawley)-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	9419406; Linked HERO ID(s): 9419406, 12162058

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Medium	The test substance source, catalogue number, lot number, and purity (>99%) was reported. 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	Medium	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 it lacks measures of variance. DCHP is not listed in the chemical information tab in the supplemental file, and raw data for results in Harlan SD rats are not provided. 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:	Hoshino, N., Iwai, M., Okazaki, Y. (2005). A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. Journal of Toxicological Sciences 30(Special):79-96.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Body weight and food intake; Hepatic/Liver: Liver weight and histology; Thyroid: Thyroid weight and histology (including parathyroid); Clinical signs: Clinical signs of toxicity; Mortality: Lethality; Renal/Kidney: Kidney weight and histology; Reproductive/Developmental: Parental animals: Weights and histology of: testes, epididymis, prostate, seminal vesicles, ovaries and uterus. Histology on vagina and mammary glands. Estrous cycle, sperm motility, numbers of homogenization-resistan spermatids in testes, number of sperm in cauda epididymal, sperm morphology. Serum levels of testosterone, LH, FSH, estradiol. Reproductive parameters and developmental parameters in offspring.;
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (10 weeks)-F0 - gestation (3 weeks)-F0- lactation (3 weeks)-F1- premating (10 weeks)-F1- mating (10 weeks)-F1 - gestation (10 weeks)-F1- lactation (10 weeks)-F0- premating (10 weeks)-F0- mating (10 weeks)-F1- premating (10 week)-F1- mating (10 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1414996

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 dicyclohexyl phthalate (DCHP; CAS NO 84-61-7). The source and purity (99.9%) were reported. Test animal species, strain, sex, age and source were reported. Initial body weight of the animals was not reported. Husbandry conditions (temperature, humidity, and light cycle) were 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 and important information are provided.
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 or consisted of initial histopathology review, and no secondary histopathology review was conducted.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Husbandry conditions were reported and similar between groups. A negative control group was included, and responses were appropriate. Decreased food intake was seen at higher concentrations of test substance in this dietary study, indicating there may have been food palatability issues thereby impacting body weight. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which have been linking to developmental and reproductive health problems. This could potentially confound results, although if control animals were exposed to the same levels, this may not substantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plastic bottles could leach phthalates that could confound results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	The study reports all parental treated animals survived. There is no indication animals were omitted from analysis.

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Study Citation:	Hoshino, N., Iwai, M., Okazaki, Y. (2005). A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. Journal of Toxicological Sciences 30(Special):79-96.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Body weight and food intake; Hepatic/Liver: Liver weight and histology; Thyroid: Thyroid weight and histology (including parathyroid); Clinical signs: Clinical signs of toxicity; Mortality: Lethality; Renal/Kidney: Kidney weight and histology; Reproductive/Developmental: Parental animals: Weights and histology of: testes, epididymis, prostate, seminal vesicles, ovaries and uterus. Histology on vagina and mammary glands. Estrous cycle, sperm motility, numbers of homogenization-resistan spermatids in testes, number of sperm in cauda epididymal, sperm morphology. Serum levels of testosterone, LH, FSH, estradiol. Reproductive parameters and developmental parameters in offspring.;
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (10 weeks)-F0 - gestation (3 weeks)-F0- lactation (3 weeks)-F1- premating (10 weeks)-F1- mating (10 weeks)-F1 - gestation (10 weeks)-F1- lactation (10 weeks)-F0- premating (10 weeks)-F0- mating (10 weeks)-F1- premating (10 week)-F1- mating (10 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1414996

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	The purity of test substance was reported as 99.9%. The study did not provide any details regarding the preparation or storage of diet containing test substance. Analytical measurements were not performed to verify concentration. The study did measure food intake and calculated daily chemical intake based on nominal concentration. Given the lack of details on preparing and analytical measurements, we cannot be confident in doses estimated.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure, frequency and duration were appropriate for study design.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Endpoints were sensitive to outcomes of interest in in agreement with OECD guideline 416.
	Metric 9:	Results presentation	Medium	Data were presented for most outcomes of interest and statistical analysis was appropriate. Not all data were reported for F1 adults (organ wt and/or histology), but lack of reporting this information is not likely to substantially impact interpretation of results.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Hoshino, N., Iwai, M., Okazaki, Y. (2005). A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. Journal of Toxicological Sciences 30(Special):79-96.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology; Endocrine: Pituitary gland and adrenal gland weight and histology; Immune/Hematological: Spleen weight and histology;
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (10 weeks)-F0 - gestation (3 weeks)-F0- lactation (3 weeks)-F1- premating (10 weeks)-F1- mating (10 weeks)-F1 - gestation (10 weeks)-F1- lactation (10 weeks)-F0- premating (10 weeks)-F0- mating (10 weeks)-F1- premating (10 week)-F1- mating (10 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1414996

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	High	This study is considered Medium for Metric 1. The test substance was identified as dicyclohexyl phthalate (DCHP; CAS NO 84-61-7). The source and purity (99.9%) were reported. Test animal species, strain, sex, age and source were reported. Initial body weight of the animals was not reported. Husbandry conditions (temperature, humidity, and light cycle) were 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 and important information are provided.
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 or consisted of initial histopathology review, and no secondary histopathology review was conducted.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	Husbandry conditions were reported and similar between groups. A negative control group was included, and responses were appropriate. Decreased food intake was seen at higher concentrations of test substance in this dietary study, indicating there may have been food palatability issues thereby impacting body weight. Animals were housed in polycarbonate cages. Polycarbonate contains BPA, which have been linking to developmental and reproductive health problems. This could potentially confound results, although if control animals were exposed to the same levels, this may not substantially impact interpretation of results. Similarly, it is unclear if glass or plastic water bottles were used. Again, plastic bottles could leach phthalates that could confound results.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	The study reports all parental treated animals survived. There is no indication animals were omitted from analysis.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Hoshino, N., Iwai, M., Okazaki, Y. (2005). A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. Journal of Toxicological Sciences 30(Special):79-96.
Health Outcome(s) and Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology; Endocrine: Pituitary gland and adrenal gland weight and histology; Immune/Hematological: Spleen weight and histology;
Duration and Exposure Route:	Oral-Diet-Duration: Reproductive/Developmental-2-F0- premating (10 weeks)-F0- mating (10 weeks)-F0 - gestation (3 weeks)-F0- lactation (3 weeks)-F1- premating (10 weeks)-F1- mating (10 weeks)-F1 - gestation (10 weeks)-F1- lactation (10 weeks)-F0- premating (10 weeks)-F0- mating (10 weeks)-F1- premating (10 week)-F1- mating (10 weeks)
Species:	Rat-Sprague-Dawley - [rat]-Both
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1414996

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	Low	The purity of test substance was reported as 99.9%. The study did not provide any details regarding the preparation or storage of diet containing test substance. Analytical measurements were not performed to verify concentration. The study did measure food intake and calculated daily chemical intake based on nominal concentration. Given the lack of details on preparing and analytical measurements, we cannot be confident in doses estimated.
	Metric 7:	Exposure timing, frequency, and duration	High	The exposure, frequency and duration were appropriate for study design.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	Endpoints were sensitive to outcomes of interest in in agreement with OECD guideline 416.
	Metric 9:	Results presentation	Low	No data are reported for organ weight or histology on brain, pituitary gland, adrenal gland or spleen in F0 generation.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Li, X., Chen, X., Hu, G., Li, L., Su, H., Wang, Y., Chen, D., Zhu, Q., Li, C., Li, J., Wang, M., Lian, Q., Ge, R. (2016). Effects of in utero exposure to dicyclohexyl phthalate on rat fetal leydig cells. International Journal of Environmental Research and Public Health 13(3):1.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Pup body weights, length, AGD, analysis of cell distribution in the testes, testicular testosterone, Leydig cell counts and cell size and nuclear size, testes immunohistochemistry and histopathology, gene expression.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	3350245

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The study included all critical information and most important information. The test substance was identified as DCHP; the source was reported, but the purity was not specified. Provided information included the test animals (Sprague Dawley) sex, and source. The starting body weights, age, and parity were not specified. Reported animal husbandry details included temperature, humidity, light cycle, number of animals per cage and details on bedding and food type. Details of test substance administration, number of animals per group, doses. Endpoint evaluation methods and quantitative results for at least one endpoint were provided.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	Low	The method of animal allocation of dams into study groups was not specified. It is unclear if animals were normalized to body weights. It was noted that three sets of fetal testes (at least one per dam) were randomly selected for downstream analysis.
	Metric 3:	Observational Bias / Blinding Changes	Medium	The study did not specify blinding; however, the concern for bias was mitigated for most of the outcomes because they were not subjective and/or were based on the use of automated/computer-driven systems, standard laboratory kits, simple objective measures (e.g., body or tissue weight), or screening-level evaluations of histopathology or quantitative immunohistochemistry.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	The study included a concurrent negative vehicle (corn oil) control. The control responses were appropriate. A positive control was not necessary for the study type. Food and water consumption were not reported in a gavage study, but the impact on results is expected to be minimal. Dam body weights were not reported. Some animal husbandry details were provided and conditions were consistent across groups. The study did not address the possibility of co-exposures to plasticizers. The cage type, vessel used for gavage, and analysis of food for contaminates were not specified. Although some details were missing, based on the information provided, there was no evidence of confounding variables.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Li, X., Chen, X., Hu, G., Li, L., Su, H., Wang, Y., Chen, D., Zhu, Q., Li, C., Li, J., Wang, M., Lian, Q., Ge, R. (2016). Effects of in utero exposure to dicyclohexyl phthalate on rat fetal leydig cells. International Journal of Environmental Research and Public Health 13(3):1.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Pup body weights, length, AGD, analysis of cell distribution in the testes, testicular testosterone, Leydig cell counts and cell size and nuclear size, testes immunohistochemistry and histopathology, gene expression.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	3350245

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Medium	Qualitative or quantitative results were reported for all specified outcomes. The study reported the number of animals per group and the sample sizes were noted either in the text or in the table/figure legends. There is no evidence of animal attrition. One figure in the study (Fig. 4) is suggestive of a 1,000 mg/kg-day dose group that is not mentioned in the methods or anywhere else in the text. It is unclear whether details of this were omitted in error, but gene expression analysis was the only endpoint in the study reporting data for this 1,000 mg/kg-day group.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Low	There are no concerns regarding the source (Sigma). The purity of the test substance was not reported in the study; based on the supplier website, the two currently available DCHP products have purities of 99% or were of analytical grade, and are certified. The test substance was not analytically verified by the testing laboratory. The reported doses are presumed to be nominal. There is no indication that concentrations in the dosing solutions were measured analytically. The test substance was dissolved in corn oil; no further details on the preparation of the test solutions (including assurance of homogeneity, frequency of preparation, or stability), or storage were provided. The gavage volume was not reported, but gavage is an appropriate route of exposure for this test substance.
	Metric 7:	Exposure timing, frequency, and duration	High	Animals were exposed daily from GD 12 to GD 21. The exposure window was justified by the study authors and was consistent with the time of Leydig cell development.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The dose spacing was not explicitly justified by the study authors, although the doses were within the range of several cited studies that also looked at reproductive effects of DCHP exposure. However, there is some lack of clarity regarding dosing. The methods and study text indicate only three dose groups at 10, 100, and 500 mg/kg-day; however, Fig. 4 is suggestive of a 1,000 mg/kg-day group. It is not clear that the lowest dose was low enough, a NOAEL was not determined. Outcome assessment methodologies were sensitive to the outcomes of interest. Sufficient details on the outcome assessment protocols were provided. Justification for the test species/strain was not provided; however, Sprague-Dawley rats were an appropriate model selection. The study used 6 animals per group which were sufficient to allow for statistical analysis. The number of testis sections examined for each endpoint was described. Testes were fixed in Bouin's solution, which is preferred over formalin, but can still cause tubular shrinkage.

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Study Citation:	Li, X., Chen, X., Hu, G., Li, L., Su, H., Wang, Y., Chen, D., Zhu, Q., Li, C., Li, J., Wang, M., Lian, Q., Ge, R. (2016). Effects of in utero exposure to dicyclohexyl phthalate on rat fetal leydig cells. International Journal of Environmental Research and Public Health 13(3):1.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Pup body weights, length, AGD, analysis of cell distribution in the testes, testicular testosterone, Leydig cell counts and cell size and nuclear size, testes immunohistochemistry and histopathology, gene expression.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 12-21)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	3350245

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	Data were adequately reported as means ± SEM, where relevant, in tables and figures. The sample sizes and statistical significance were noted. The methods of statistical analysis were reported and were appropriate for most datasets. It was not specified whether the litter endpoints (litter size, number of pups, sex ratios etc.,) were analyzed using the litter as the unit of statistical analysis. Individual animal data were not provided.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Uterine weight, number of implantation sites, resorption, dead and live fetuses, and corpora lutea. Live fetuses weight, sex, anogenital distance, external, visceral and skeletal abnormalities, and the degree of trans-abdominal testicular migration.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (dicyclohexyl phthalate [DCHP] with CAS RN (84-61-7)). The source and purity of the test substance were reported. Test animal species, strain, sex, and initial body weights were reported. Females were primiparous, age was not reported. The source of the animals was provided. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were reported. Food and water availability were available 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	"Mated females were randomly assigned to treatment groups by stratified randomization so that the mean body weights on GD 0 did not differ among treatment groups."
	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 or assessing fetal abnormalities.
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 reported and were consistent between groups. However, 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 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. Animals were housed in polycarbonate cages. Food intake was reported and was, at time decreased during the study, however overall food intake throughout the entire study period was similar to controls.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. Quantitative outcomes were reported for each group for most outcomes (number of corpora lutea not reported). There is no indication of attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Uterine weight, number of implantation sites, resorption, dead and live fetuses, and corpora lutea. Live fetuses weight, sex, anogenital distance, external, visceral and skeletal abnormalities, and the degree of trans-abdominal testicular migration.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The source and purity (99%) of the test substance were reported. Gavage volume was reported to be 10 ml/kg. This is within the recommended gavage volume, although on the upper limit. The study authors report this higher volume was necessary due to the limited solubility of the test substance in oil. Dosing solutions were analyzed by gas chromatography and study authors report findings indicated solutions were stable for up to 14 days when stored in dark place at room temperature (data not shown). Preparation was based on these results. Doses were adjusted based on changes in body weights throughout the study period.
	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 species was appropriate for study's aim. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on previously reported findings. Three dose levels were included and are sufficient . Assessment of endpoints were appropriate. 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	Quantitative data were presented as means +/- SD for results. Statistical tests were reported and appropriate. The litter was used as the statistical unit.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Mortality: Mortality; Nutritional/Metabolic: Body weight, body weight gain and food intake;
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (dicyclohexyl phthalate [DCHP] with CAS RN (84-61-7)). The source and purity of the test substance were reported. Test animal species, strain, sex, and initial body weights were reported. Females were primiparous, age was not reported. The source of the animals was provided. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were reported. Food and water availability were available 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	"Mated females were randomly assigned to treatment groups by stratified randomization so that the mean body weights on GD 0 did not differ among treatment groups."
	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, body weight, food intake).
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 reported and were consistent between groups. However, 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 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. Animals were housed in polycarbonate cages. Lack of controlling for plasticizers is not likely to substantially impact results. Food intake was reported and was, at time decreased during the study, however overall food intake throughout the entire study period was similar to controls.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. Quantitative outcomes were reported for each group for outcomes. There is no indication of attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Mortality: Mortality; Nutritional/Metabolic: Body weight, body weight gain and food intake;
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain		Metric	Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The source and purity (99%) of the test substance were reported. Gavage volume was reported to be 10 ml/kg. This is within the recommended gavage volume, although on the upper limit. The study authors report this higher volume was necessary due to the limited solubility of the test substance in oil. Dosing solutions were analyzed by gas chromatography and study authors report findings indicated solutions were stable for up to 14 days when stored in dark place at room temperature (data not shown). Preparation was based on the stability results. Doses were adjusted based on changes in body weights throughout the study period.
	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 species was appropriate for study's aim. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on previously reported findings. Three dose levels were included and are sufficient . Assessment of endpoints were appropriate. Outcomes were assessed consistently across study groups. There are no major concerns regarding the outcome methodology for outcomes of interest.
	Metric 9:	Results presentation	High	Quantitative data for body weight gain and food intake were presented as means +/- SD for results. Statistical tests were reported and appropriate. The study reported no animals died in text.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Clinical signs: Clinical signs of toxicity
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (dicyclohexyl phthalate [DCHP] with CAS RN (84-61-7)). The source and purity of the test substance were reported. Test animal species, strain, sex, and initial body weights were reported. Females were primiparous, age was not reported. The source of the animals was provided. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were reported. Food and water availability were available 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	"Mated females were randomly assigned to treatment groups by stratified randomization so that the mean body weights on GD 0 did not differ among treatment groups."
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, clinical signs of toxicity do not require blinding.
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 reported and were consistent between groups. However, 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 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. Animals were housed in polycarbonate cages. Lack of controlling for plasticizers is not likely to substantially impact results. Food intake was reported and was, at time decreased during the study, however overall food intake throughout the entire study period was similar to controls.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	High	All animals were accounted for in the results. Qualitative outcomes were reported. There is no indication of attrition.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Clinical signs: Clinical signs of toxicity
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The source and purity (99%) of the test substance were reported. Gavage volume was reported to be 10 ml/kg. This is within the recommended gavage volume, although on the upper limit. The study authors report this higher volume was necessary due to the limited solubility of the test substance in oil. Dosing solutions were analyzed by gas chromatography and study authors report findings indicated solutions were stable for up to 14 days when stored in dark place at room temperature (data not shown). Preparation was in accordance with these results. Doses were adjusted based on changes in body weights throughout the study period.
	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 species was appropriate for study's aim. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on previously reported findings. Three dose levels were included and are sufficient. Study authors report that clinical signs were assessed daily, however no other details were provided (e.g. cage-side observation).
	Metric 9:	Results presentation	Medium	Clinical signs were reported as negative in text. Statistical analysis was not necessary for negative data.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Body weight, body weight gain and food intake; Hepatic/Liver: Serum AST, ALT, cholesterol, and triglycerides, liver weight, liver histopathology, and liver enzyme activity (cyanide-insensitive palmitoyl CoA oxidase and peroxisomal β-oxidation).;
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	The chemical was identified by name (dicyclohexyl phthalate [DCHP] with CAS RN (84-61-7). The source and purity of the test substance were reported. Test animal species, strain, sex, and initial body weights were reported. Females were primiparous, age was not reported. The source of the animals was provided. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were reported. Food and water availability were available 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	"Mated females were randomly assigned to treatment groups by stratified randomization so that the mean body weights on GD 0 did not differ among treatment groups."
	Metric 3:	Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, endpoints were either non subjective in nature or histopathology.
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 reported and were consistent between groups. However, 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 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. Animals were housed in polycarbonate cages. Lack of controlling for plasticizers is not likely to substantially impact results. Food intake was not reported.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Medium	The number of animals treated and reported in results was a range (6-9/group). While it was reported that no animals died, attrition was not specified. Because the number of animals is presented as a range, it is difficult to know if any were excluded from analysis.
Domain 5: Exposure Methods Sensitivity				

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Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Body weight, body weight gain and food intake; Hepatic/Liver: Serum AST, ALT, cholesterol, and triglycerides, liver weight, liver histopathology, and liver enzyme activity (cyanide-insensitive palmitoyl CoA oxidase and peroxisomal β-oxidation).;
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The source and purity (99%) of the test substance were reported. Gavage volume was reported to be 10 ml/kg. This is within the recommended gavage volume, although on the upper limit. The study authors report this higher volume was necessary due to the limited solubility of the test substance in oil. Dosing solutions were analyzed by gas chromatography and study authors report findings indicated solutions were stable for up to 14 days when stored in dark place at room temperature (data not shown). Doses were adjusted based on changes in body weights throughout the study period.
	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 species was appropriate for study's aim. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on previously reported findings. Three dose levels were included and are sufficient to determine a LOEL, but an additional lower dose would be required to determine a NOEL. Hepatic outcomes assessed were sensitive to determine hepatic effects. Terminal body weights were assessed appropriately (adjusted for uterine weight).
	Metric 9:	Results presentation	Medium	Most hepatic endpoints were reported as mean +/- SD. Hepatic cyanide-insensitive palmitoyl CoA oxidase activity was not reported. Histopathology was reported as negative in text.Terminal body weight data was reported as BW minus uterine weight. Body weight data that includes the uterine weight was not reported. Statistical methods were reported and appropriate.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Uterine weight, number of implantation sites, resorption, dead and live fetuses, and corpora lutea. Live fetuses weight, sex, anogenital distance, external, visceral and skeletal abnormalities, and the degree of trans-abdominal testicular migration.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric	Rating	Comments
Domain 1: Reporting Quality			
	Metric 1: Reporting Quality	Medium	The chemical was identified by name (dicyclohexyl phthalate [DCHP] with CAS RN (84-61-7). The source and purity of the test substance were reported. Test animal species, strain, sex, and initial body weights were reported. Females were primiparous, age was not reported. The source of the animals was provided. Husbandry conditions (temperature, humidity, light cycle, animals/cage) were not reported. Cage and bedding type were reported. Food and water availability were available 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	"Mated females were randomly assigned to treatment groups by stratified randomization so that the mean body weights on GD 0 did not differ among treatment groups."
	Metric 3: Observational Bias / Blinding Changes	Medium	Blinding or other measures to reduce observational bias were not reported; however, endpoint was not subjective in nature (uterine 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 were reported and were consistent between groups. However, 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 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. Animals were housed in polycarbonate cages. Lack of controlling for plasticizers is not likely to substantially impact results.
Domain 4: Selective Reporting and Attrition			
	Metric 5: Selective Reporting and Attrition	Medium	The number of animals treated and reported in results was a range (6-9/group). While it was reported that no animals died, attrition was not reported. Because the number of animals is presented as a range, it is difficult to know if any were excluded from analysis.
Domain 5: Exposure Methods Sensitivity			

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Study Citation:	Saillenfait, A. M., Gallissot, F., Sabate, J. P. (2009). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology 29(6):510-521.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Uterine weight, number of implantation sites, resorption, dead and live fetuses, and corpora lutea. Live fetuses weight, sex, anogenital distance, external, visceral and skeletal abnormalities, and the degree of trans-abdominal testicular migration.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-20)
Species:	Rat-Sprague-Dawley - [rat]-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1465017

Domain	Metric		Rating	Comments
	Metric 6:	Chemical administration and characterization	High	The source and purity (99%) of the test substance were reported. Gavage volume was reported to be 10 ml/kg. This is within the recommended gavage volume, although on the upper limit. The study authors report this higher volume was necessary due to the limited solubility of the test substance in oil. Dosing solutions were analyzed by gas chromatography and study authors report findings indicated solutions were stable for up to 14 days when stored in dark place at room temperature (data not shown). Preparation was noted to be in accordance with stability results. Doses were adjusted based on changes in body weights throughout the study period.
	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	Low	No guideline was specified. The species was appropriate for study's aim. The number of animals/group was appropriate as group sizes were large enough and sufficient for statistical analysis. The doses were chosen based on previously reported findings. Three dose levels were included and are sufficient to determine a LOEL, but an additional lower dose would be required to determine a NOEL. Uterine weight was measured at the time of sacrifice and was appropriate for the purposes of correcting terminal body weight measurements.
	Metric 9:	Results presentation	Uninformative	Uterine weights were not reported, only body weights corrected for uterine weights were reported.

Additional Comments: None

Overall Quality Determination Uninformative

Study Citation:	Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicology Letters 189(1):14-20.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Endpoints assessed in F0 dams: number of litters, gestation index (%), gestation length (days), number of pups born, delivery index (%), birth index (%) and live birth index (%), number of implantation sites and absolute and relative ovary weights. Endpoints assessed in F1 offspring: ventral surface observations for abnormalities, timing of preputial separation or vaginal patency, anogenital distance, nipple retention, estrous cycle timing, sex ratio on PND 0, number of live pups on PND4 and 21, viability index on PND4 (%), weaning index on PND 21 (%), body weights, gross necropsy (for ectopic/atrophic testes, agenesis of the gubnernaculums, epididymides, sex accessory gland and epididymal granulomas) absolute and relative organ weights (testis, epididymis, ventral prostate, seminal vesicle, levator ani-bulbocavernous muscle, ovary, uterus, brain, pituitary, thyroid, adrenal, kidney, liver), and histopathology (liver, kidneys, testes, epididymides, uterus, ovaries, vagina, pituitary, thyroid) and clinical signs of toxicity.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
Species:	Rat-Other (Crl:CD (SD))-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1061309

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information is reported. Test chemical name, CASRN, purity and source are reported. Test animal species, strain, sex, starting age, commercial source and animal housing conditions (including temperature, humidity, day/night cycle, food and water availability and number of animals per cage) were all reported. Starting animal body weights were not reported and the authors did not show data on maternal body weights that could contain this information. The experimental procedure and endpoint assessment methods were described in adequate detail.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were divided into groups using a body weight stratification randomization method.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were reported. Endpoints were generally not subjective in nature or included initial review of histopathology, so the influence of observational bias is likely low.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Low	An appropriate negative control was included and there was no response in the control group. Not all information is reported to determine the influence of confounding factors. Animals were housed in stainless steel wire mesh cages; however, the authors do not describe whether precautions were taken to eliminate exposure to trace amounts of endocrine-disrupting chemicals from other animal housing materials or food and water. The authors did cull litters to the same size in order to reduce potential differences in nutritional intake between different groups. Food and water intake was not recorded in a gavage study. Animal husbandry conditions were comparable across groups.
Domain 4: Selective Reporting and Attrition				

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Study Citation:	Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicology Letters 189(1):14-20.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Endpoints assessed in F0 dams: number of litters, gestation index (%), gestation length (days), number of pups born, delivery index (%), birth index (%) and live birth index (%), number of implantation sites and absolute and relative ovary weights. Endpoints assessed in F1 offspring: ventral surface observations for abnormalities, timing of preputial separation or vaginal patency, anogenital distance, nipple retention, estrous cycle timing, sex ratio on PND 0, number of live pups on PND4 and 21, viability index on PND4 (%), weaning index on PND 21 (%), body weights, gross necropsy (for ectopic/atrophic testes, agenesis of the gubnernaculums, epididymides, sex accessory gland and epididymal granulomas) absolute and relative organ weights (testis, epididymis, ventral prostate, seminal vesicle, levator ani-bulbocavernous muscle, ovary, uterus, brain, pituitary, thyroid, adrenal, kidney, liver), and histopathology (liver, kidneys, testes, epididymides, uterus, ovaries, vagina, pituitary, thyroid) and clinical signs of toxicity.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
Species:	Rat-Other (Crl:CD (SD))-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1061309

Domain	Metric		Rating	Comments
	Metric 5:	Selective Reporting and Attrition	Low	One animal died early from dystocia during the study, but this omission was explained by the authors. Most prespecified outcomes are described in the results, however, results for gross necropsy (in dams or F1 offspring), clinical signs, organ weights for ovary, thyroid and kidney in F0 dams, and organ weight data in mated F1 groups were omitted; the omission of these data are not explained. Additionally, data were not shown for several endpoints with treatment-related findings; only limited qualitative statements were provided. Histopathology results for most organs were not reported. The sample sizes for many of these endpoints cannot be determined.
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance identity and source (Wako Pure Chemical Industries) and purity (99.9%) were reported. It is unclear whether the authors performed independent analytical verification of the test substance purity, but they did describe taking steps to verify the test substance concentration and stability in the vehicle. Test substance storage and preparation conditions were not described, but the test chemical is likely to have no concerns regarding stability. A gavage volume of 2 mL/kg was reported and appropriate. Administered doses are only reported nominally, but reported doses are likely to be accurate.
	Metric 7:	Exposure timing, frequency, and duration	Medium	Animals were exposed from GD6 – LD20. This window was appropriate for most of the endpoints assessed. However, an Implantation index was included as an endpoint, which would warrant exposures starting on GD5.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The species and strain were appropriate to measure endpoints of interest. Doses were chosen based on a preliminary study, and cover the full range of responses, including a NOAEL, LOAEL. There were no concerns regarding endpoint assessment methods. There may be a slight concern that some endpoints in F1 offspring were measured at 10 weeks of age (~2 months post exposure during lactation), but the authors intended to measure the long-lasting impacts of test chemical exposure, so this aspect of their experimental design is justified. The number of animals per group (10 dams/group) is lower than guideline recommendations for typical prenatal exposure studies (OECD TG 414), which recommend at least 20 females. Sample sizes (when reported) were adequate to assess the endpoints of interest.

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Study Citation:	Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicology Letters 189(1):14-20.
Health Outcome(s) and Reported Health Effect(s):	Reproductive/Developmental: Endpoints assessed in F0 dams: number of litters, gestation index (%), gestation length (days), number of pups born, delivery index (%), birth index (%) and live birth index (%), number of implantation sites and absolute and relative ovary weights. Endpoints assessed in F1 offspring: ventral surface observations for abnormalities, timing of preputial separation or vaginal patency, anogenital distance, nipple retention, estrous cycle timing, sex ratio on PND 0, number of live pups on PND4 and 21, viability index on PND4 (%), weaning index on PND 21 (%), body weights, gross necropsy (for ectopic/atrophic testes, agenesis of the gubnernaculums, epididymides, sex accessory gland and epididymal granulomas) absolute and relative organ weights (testis, epididymis, ventral prostate, seminal vesicle, levator ani-bulbocavernous muscle, ovary, uterus, brain, pituitary, thyroid, adrenal, kidney, liver), and histopathology (liver, kidneys, testes, epididymides, uterus, ovaries, vagina, pituitary, thyroid) and clinical signs of toxicity.
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
Species:	Rat-Other (Crl:CD (SD))-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1061309

Domain	Metric	Rating	Comments
	Metric 9: Results presentation	Medium	The litter is the unit of sampling for endpoints measured in F1 offspring prior to weaning. Quantitative results with means, standard deviation, and sample sizes are reported for most endpoints. Some negative data is described qualitatively, but results for some endpoints were not reported (see Metric 4). Incidence data for histopathology in F1 offspring is omitted and biologically relevant effects are described qualitatively, which may have a minor impact on the results. Statistical methods are described in adequate detail and are appropriate to analyze endpoints of interest.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicology Letters 189(1):14-20.
Health Outcome(s) and Reported Health Effect(s):	Mortality: Death
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
Species:	Rat-Other (Crl:CD (SD))-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1061309

Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information is reported. Test chemical name, CASRN, purity and source are reported. Test animal species, strain, sex, starting age, commercial source and animal housing conditions (including temperature, humidity, day/night cycle, food and water availability and number of animals per cage) were all reported. Starting animal body weights were not reported and the authors did not show data on maternal body weights that could contain this information. Parity was not specified. The experimental procedure and endpoint assessment methods were described in adequate detail.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were divided into groups using a body weight stratification randomization method.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were reported. Endpoints were generally not subjective in nature or included initial review of histopathology, so the influence of observational bias is likely low.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An appropriate negative control was included and there was no response in the control group. Not all information is reported to determine the influence of confounding factors. Animals were housed in stainless steel wire mesh cages; however, the authors do not describe whether precautions were taken to eliminate exposure to trace amounts of endocrine-disrupting chemicals from other animal housing materials or food and water. The authors did cull litters to the same size in order to reduce potential differences in nutritional intake between different groups. Food and water intake was not recorded in a gavage study. Animal husbandry conditions were comparable across groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	One animal died early from dystocia during the study, but this omission was explained by the authors. Most prespecified outcomes are described in the results, however, results for gross necropsy (in dams or F1 offspring), clinical signs, organ weights for ovary, thyroid and kidney in F0 dams, and organ weight data in mated F1 groups were omitted; the omission of these data are not explained. Additionally, data were not shown for several endpoints with treatment-related findings; only limited qualitative statements were provided. Histopathology results for most organs were not reported. The sample sizes for many of these endpoints cannot be determined.

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Study Citation:	Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicology Letters 189(1):14-20.
Health Outcome(s) and Reported Health Effect(s):	Mortality: Death
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
Species:	Rat-Other (Crl:CD (SD))-Female
Chemical:	Dicyclohexyl Phthalate- Parent compound
HERO ID:	1061309

Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance identity and source (Wako Pure Chemical Industries) and purity (99.9%) were reported. It is unclear whether the authors performed independent analytical verification of the test substance purity, but they did describe taking steps to verify the test substance concentration and stability in the vehicle. Test substance storage and preparation conditions were not described, but the test chemical is likely to have no concerns regarding stability. A gavage volume of 2 mL/kg was reported and appropriate. Administered doses are only reported nominally, but reported doses are likely to be accurate.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure were appropriate for these endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species and strain were appropriate to measure endpoints of interest. Doses were chosen based on a preliminary study, and cover the full range of responses, including a NOAEL, LOAEL. There were no concerns regarding endpoint assessment methods. The frequency of animal observations was reported. The sample size was appropriate for the endpoint.
	Metric 9:	Results presentation	High	Mortality results were adequately reported.

Additional Comments: None

Overall Quality Determination Medium

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Health Outcome(s) and Reported Health Effect(s):	Nutritional/Metabolic: Maternal body weights (GD 0, 6, 13, 20, PND 4, 7, 14, 21); Hepatic/Liver: Absolute and relative liver weights (F0 dams).;
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
Species:	Rat-Other (Crl:CD (SD))-Female
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Domain	Metric		Rating	Comments
Domain 1: Reporting Quality				
	Metric 1:	Reporting Quality	Medium	All critical information is reported. Test chemical name, CASRN, purity and source are reported. Test animal species, strain, sex, starting age, commercial source and animal housing conditions (including temperature, humidity, day/night cycle, food and water availability and number of animals per cage) were all reported. Starting animal body weights were not reported and the authors did not show data on maternal body weights that could contain this information. Parity was not specified. The experimental procedure and endpoint assessment methods were described in adequate detail.
Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were divided into groups using a body weight stratification randomization method.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were reported. Endpoints were generally not subjective in nature or included initial review of histopathology, so the influence of observational bias is likely low.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An appropriate negative control was included and there was no response in the control group. Not all information is reported to determine the influence of confounding factors. Animals were housed in stainless steel wire mesh cages; however, the authors do not describe whether precautions were taken to eliminate exposure to trace amounts of endocrine-disrupting chemicals from other animal housing materials or food and water. The authors did cull litters to the same size in order to reduce potential differences in nutritional intake between different groups. Food and water intake was not recorded in a gavage study. Animal husbandry conditions were comparable across groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	One animal died early from dystocia during the study, but this omission was explained by the authors. Most prespecified outcomes are described in the results, however, results for gross necropsy (in dams or F1 offspring), clinical signs, organ weights for ovary, thyroid and kidney in F0 dams, and organ weight data in mated F1 groups were omitted; the omission of these data are not explained. Additionally, data were not shown for several endpoints with treatment-related findings; only limited qualitative statements were provided. Histopathology results for most organs were not reported. The sample sizes for many of these endpoints cannot be determined.

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Domain	Metric		Rating	Comments
Domain 5: Exposure Methods Sensitivity				
	Metric 6:	Chemical administration and characterization	Medium	The test substance identity and source (Wako Pure Chemical Industries) and purity (99.9%) were reported. It is unclear whether the authors performed independent analytical verification of the test substance purity, but they did describe taking steps to verify the test substance concentration and stability in the vehicle. Test substance storage and preparation conditions were not described, but the test chemical is likely to have no concerns regarding stability. A gavage volume of 2 mL/kg was reported and appropriate. Administered doses are only reported nominally, but reported doses are likely to be accurate.
	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure were appropriate for these endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	High	The species and strain were appropriate to measure endpoints of interest. Doses were chosen based on a preliminary study, and cover the full range of responses, including a NOAEL, LOAEL. There were no concerns regarding endpoint assessment methods. The frequency of animal body weight measurements was reported; organs (absolute and relative) were weighed at termination. Histopathology was not conducted, but histopathology is often not conducted in dams in prenatal toxicity studies, and hepatotoxicity was not a focus of the study. The sample size was appropriate for the endpoint.
	Metric 9:	Results presentation	Medium	Negative findings (no change) for dam body weights were qualitatively reported. Terminal body weights were not reported despite measurements of organ weights. The study provided quantitative relative liver data. Absolute liver weight results were not reported but were stated as an endpoint in the methods.

Additional Comments: None

Overall Quality Determination Medium

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Health Outcome(s) and Reported Health Effect(s):	Clinical Signs: Clinical observations of toxicity; Thyroid: Absolute and relative thyroid weights (F0 dams).; Renal/Kidney: Absolute and relative Kidney weights (F0 dams).;
Duration and Exposure Route:	Oral-Gavage-Duration: Reproductive/Developmental-1-F0 - gestation (GD 6-GD 20)-F0- lactation (PND 0-PND 20)
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Domain 2: Selection and Performance				
	Metric 2:	Allocation	High	Animals were divided into groups using a body weight stratification randomization method.
	Metric 3:	Observational Bias / Blinding Changes	Medium	No measures to reduce observational bias were reported. Endpoints were generally not subjective in nature or included initial review of histopathology, so the influence of observational bias is likely low.
Domain 3: Confounding / Variable Control				
	Metric 4:	Confounding / Variable Control	Medium	An appropriate negative control was included and there was no response in the control group. Not all information is reported to determine the influence of confounding factors. Animals were housed in stainless steel wire mesh cages; however, the authors do not describe whether precautions were taken to eliminate exposure to trace amounts of endocrine-disrupting chemicals from other animal housing materials or food and water. The authors did cull litters to the same size in order to reduce potential differences in nutritional intake between different groups. Food and water intake was not recorded in a gavage study. Animal husbandry conditions were comparable across groups.
Domain 4: Selective Reporting and Attrition				
	Metric 5:	Selective Reporting and Attrition	Low	One animal died early from dystocia during the study, but this omission was explained by the authors. Most prespecified outcomes are described in the results, however, results for gross necropsy (in dams or F1 offspring), clinical signs, organ weights for ovary, thyroid and kidney in F0 dams, and organ weight data in mated F1 groups were omitted; the omission of these data are not explained. Additionally, data were not shown for several endpoints with treatment-related findings; only limited qualitative statements were provided. Histopathology results for most organs were not reported. The sample sizes for many of these endpoints cannot be determined.

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	Metric 7:	Exposure timing, frequency, and duration	High	The timing, duration, and frequency of the exposure were appropriate for these endpoints of interest.
Domain 6: Outcome Measures and Results Display				
	Metric 8:	Endpoint sensitivity and specificity	Medium	The species and strain were appropriate to measure endpoints of interest. Doses were chosen based on a preliminary study, and cover the full range of responses, including a NOAEL, LOAEL. There were no concerns regarding endpoint assessment methods. Organs (absolute and relative) were weighed at termination. Histopathology was not conducted, but histopathology is often not conducted in dams in prenatal toxicity studies. The frequency of animal observations was reported, but the nature of the observations (cage-side or detailed clinical) were not specified. The sample size was appropriate for the endpoint.
	Metric 9:	Results presentation	Uninformative	Results for these endpoints were omitted from the study report.

Additional Comments: None

Overall Quality Determination Uninformative