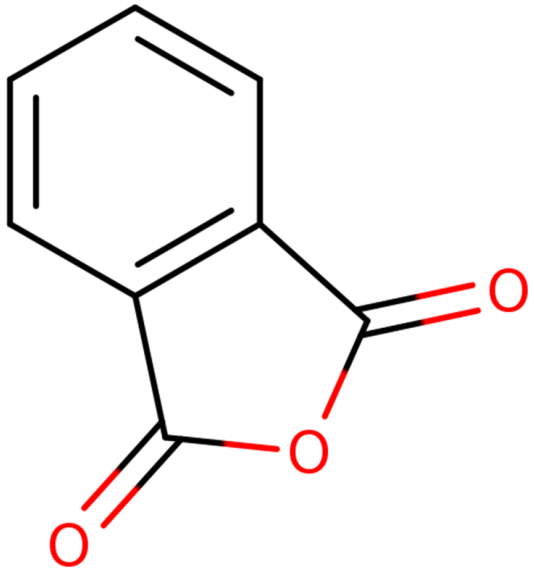
United States
Environmental Protection Agency

March 2026
Office of Chemical Safety and
Pollution Prevention

Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Phthalic Anhydride

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 85-44-9



March 2026

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

Table of Contents

Metabolite: Phthalic acid

Short-term (>1-30 days)

Other (specify)

Metabolite: 1,2-phthalic acid

Short-term (>1-30 days)

Study Citation:	AG,, Bayer (1978). [Acute oral toxicity - phthalic anhydride (Unpublished short report)].
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) Acute oral rat
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353568

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by name as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A negative or vehicle control is not required for acute oral lethality studies.
	Metric 5:	Positive Controls	N/A	Positive controls are not required for acute oral lethality studies.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test substance was diluted in DM50. No further details on preparation and storage of the test substance were provided. Animals were administered one dose, so frequency details are not needed.
	Metric 8:	Consistency of Exposure Administration	Low	The gavage volumes were not clearly reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Doses were clearly reported. Animals were administered single nominal doses of the test substance diluted in a dm50 vehicle. The concentrations in the test solutions were not verified; however, given the single-dose preparation required, this is unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	Animals were administered a single dose, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose spacing sufficiently covered the full range of responses.
	Metric 12:	Exposure Route and Method	High	Animals were dosed via gavage, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male Wister II rats. Initial body weights were reported. The rats were "of the breeder Winkelmann;" it is unclear if this was the source. Age was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Test animals were housed 5 animals per cage. No other husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 10 animals per group.
Domain 5: Outcome Assessment				

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Study Citation:	AG,, Bayer (1978). [Acute oral toxicity - phthalic anhydride (Unpublished short report)].
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) Acute oral rat
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353568

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	Animals were observed for mortality for 14 days. The frequency of observations was not specified, but the times of death were reported, so it appears that animals were observed daily.
	Metric 17:	Consistency of Outcome Assessment	Medium	There is no indication of inconsistencies across dosing groups. Although details are limited, the duration of observations was the same for all groups.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were monitored for survival.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	N/A	The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment. Body weights were not recorded.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	The statistical method used to calculate LC50 was reported and appropriate.
	Metric 24:	Reporting of Data	High	Mortality data were presented for each test group.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	AG,, Bayer (1978). [Acute oral toxicity - phthalic anhydride (Unpublished short report)].
Health Outcome(s):	Clinical signs (Clinical signs)
Reported Health Effect(s):	Clinical signs (Clinical signs): Clinical signs
Duration:	Acute (less than or equal to 24 hr) Acute oral rat
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353568

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by name as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A negative or vehicle control is not required for acute oral lethality studies.
	Metric 5:	Positive Controls	N/A	Positive controls are not required for acute oral lethality studies.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test substance was diluted in DM50. No further details on preparation and storage of the test substance were provided. Animals were administered one dose, so frequency details are not needed.
	Metric 8:	Consistency of Exposure Administration	Low	The gavage volumes were not clearly reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Doses were clearly reported. Animals were administered a single nominal dose of the test substance diluted in Dm50. The concentration in the test solution was not verified; this is unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	Animals were administered a single dose, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose spacing sufficiently covered the full range of responses.
	Metric 12:	Exposure Route and Method	High	Animals were dosed via gavage, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male Wister II rats. Initial body weights were reported. Animals were ”of the breeder Winkelmann;” it is unclear if this is the source. Age was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Test animals were housed 5 animals per cage. No other husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 10 animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Limited details are provided, but animals were observed for clinical signs of toxicity for 14 days. The nature (e.g., cage side or detailed clinical) and frequency of observations were not specified.

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Study Citation:	AG,, Bayer (1978). [Acute oral toxicity - phthalic anhydride (Unpublished short report)].
Health Outcome(s):	Clinical signs (Clinical signs)
Reported Health Effect(s):	Clinical signs (Clinical signs): Clinical signs
Duration:	Acute (less than or equal to 24 hr) Acute oral rat
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353568

Domain	Metric		Rating	Comments
	Metric 17:	Consistency of Outcome Assessment	Low	Other than the overall duration of observations, insufficient data are provided to determine whether there was consistency of outcome assessment.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were monitored for toxicological symptoms.
	Metric 19:	Blinding of Assessors	Medium	The study did not report whether assessors were blinded to treatment group for evaluating clinical signs, and this is not likely to have a substantial impact on results.
	Metric 20:	Negative Control Response	N/A	The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis of clinical signs was not necessary.
	Metric 24:	Reporting of Data	Medium	The number of animals with clinical signs was reported for each test group; however, it was not specified which, or how many, animals exhibited each of the clinical signs mentioned.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Biomedical,, Exxon (1988). Primary dermal irritation study in the rabbit (Test material: MRD-87-113).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Dermal irritation
Duration:	Acute (less than or equal to 24 hr) 4 hours dermal irritaiton study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980183

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was reported in this reference as MRD-87-113. The cover page contains the code 88MRL 34. This code appears in a companion document (HERO 13028219), which identifies it as the test substance, phthalic anhydride.
	Metric 2:	Test Substance Source	High	The test substance was supplied by the Sponsor (Exon Mobile; Batch No. 1)
	Metric 3:	Test Substance Purity	Low	The report states: "The test material was a black solid which was assumed to be 100% pure for the purpose of dosing."
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	OECD 404 TG do not require separate groups of control animals. Untreated skin on each animal may serve as a control.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were selected using a random number sorting procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test material was a solid. The test material was applied either neat or moistened with saline. Details on preparing the solid for application were not provided (e.g., it is not reported if the material was ground or how fine the particles were). For the moistened group, 0.5 ml of saline was added to 0.5 grams of test material for application. Storage of the test substance was not reported; however, since the test substance was applied once, this is not expected to have a significant impact on the study results.
	Metric 8:	Consistency of Exposure Administration	Medium	The surface area of the application site was not reported. The weight (0.5g) or volume (0.5mL) applied and the manner in which the sites were treated (use of a gauze patch secured with tape) were consistent
	Metric 9:	Reporting of Doses/Concentrations	Medium	The dose was clearly reported. The test substance was applied neat (0.5g). The body weights of the rabbits on the day of application were reported. There is some uncertainty of dose because the purity and composition of the test substance are not known.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration and frequency were appropriate and agreed with OECD guidelines.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	A single 0.5g dose of solid test material was tested as per OECD TG.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.

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Study Citation:	Biomedical,, Exxon (1988). Primary dermal irritation study in the rabbit (Test material: MRD-87-113).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Dermal irritation
Duration:	Acute (less than or equal to 24 hr) 4 hours dermal irritaiton study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980183

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was appropriate (3/sex).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive to endpoint of interest (Draize Method of Scoring).
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rabbits.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, outputs were either not subjective (death, weights) or not required per OECD guidelines.
	Metric 20:	Negative Control Response	N/A	The negative control area was not included.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Animals were exposed to the neat test substance and then, 7 days later, exposed to the moistened test substance in a different area. This is a deviation from OECD guidelines. There is uncertainty whether the reaction to the second application of the moistened test substance may have been the result of a sensitization and not irritation.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Irritation study, statistical analysis is not required.
	Metric 24:	Reporting of Data	High	Data are fully reported for each animal.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Biomedical,, Exxon (1988). Primary dermal irritation study in the rabbit (Test material: MRD-87-113).
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) 4 hours dermal irritaiton study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980183

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was reported in this reference as MRD-87-113. The companion document (HERO 13028219) identified the test substance as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The test substance was supplied by the Sponsor (Exon Mobile; Batch No. 1)
	Metric 3:	Test Substance Purity	Low	The report states: "The test material was a black solid which was assumed to be 100% pure for the purpose of dosing."
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	OECD 404 TG does not require separate groups of control animals. Untreated skin on each animal may serve as the control.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were selected using a random number sorting procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test material was a solid. The test material was applied either neat or moistened with saline. Details on preparing the solid for application were not provided (e.g., it is not reported if the material was ground or how fine the particles were). For the moistened group, 0.5 ml of saline was added to 0.5 grams of test material for application. Storage of the test substance was not reported; however, since the test substance was applied once, this is not expected to have a significant impact on the study results.
	Metric 8:	Consistency of Exposure Administration	Medium	The surface area of the application site was not reported. The weight (0.5g) or volume (0.5mL) applied and the manner in which the sites were treated (use of a gauze patch secured with tape) were consistent.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The dose was clearly reported. The test substance was applied neat (0.5g). The body weights of the rabbits on the day of application were reported. There is some uncertainty of the dose because the purity and composition of the test substance are not known.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration and frequency were appropriate and agreed with OECD guidelines.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	A single 0.5g dose of solid test material was tested as per OECD TG.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was appropriate (3/sex).

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Study Citation:	Biomedical,, Exxon (1988). Primary dermal irritation study in the rabbit (Test material: MRD-87-113).
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) 4 hours dermal irritaiton study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980183

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive to endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rabbits.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, outputs were either not subjective (death, weights) or not required per OECD guidelines.
	Metric 20:	Negative Control Response	N/A	A separate group of negative control animals is not included in this study type.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Animals were exposed to the neat test substance and then 7 days later exposed to moistened test substance in a different area. This is a deviation of OECD guidelines. There is uncertainty if the reaction to the second application of moistened test substance may have been the result of a sensitization and not irritation. However, the applicaiton method is not a confounding factor for this outcome (mortality).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis is not possible since there is not a control group.
	Metric 24:	Reporting of Data	High	Data are fully reported for each animal.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Biomedical,, Exxon (1988). Primary dermal irritation study in the rabbit (Test material: MRD-87-113).
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Acute (less than or equal to 24 hr) 4 hours dermal irritaiton study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980183

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was reported in this reference as MRD-87-113. The cover page contains the code 88MRL 34. This code appears in a companion document (HERO 13028219), which identifies it as the test substance, phthalic anhydride.
	Metric 2:	Test Substance Source	High	The test substance was supplied by the Sponsor (Exon Mobile; Batch No. 1)
	Metric 3:	Test Substance Purity	Low	The report states: "The test material was a black solid which was assumed to be 100% pure for the purpose of dosing."
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	OECD 404 TG does not require separate groups of control animals. Untreated skin on each animal may serve as the control.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were selected using a random number sorting procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test material was a solid. The test material was applied either neat or moistened with saline. Details on preparing the solid for application were not provided (e.g., it is not reported if the material was ground or how fine the particles were). For the moistened group, 0.5 ml of saline was added to 0.5 grams of test material for application. Storage of the test substance was not reported; however, since the test substance was applied once, this is not expected to have a significant impact on the study results.
	Metric 8:	Consistency of Exposure Administration	Medium	The surface area of the application site was not reported. The weight (0.5g) or volume (0.5mL) applied and the manner in which the sites were treated (use of a gauze patch secured with tape) were consistent.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The dose was clearly reported. The test substance was applied neat (0.5g). The body weights of the rabbits on the day of application were reported. There is some uncertainty of dose because the purity and composition of the test substance are not known.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration and frequency were appropriate and agreed with OECD guidelines.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	A single 0.5g dose of solid test material was tested as per OECD TG.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.

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Study Citation:	Biomedical,, Exxon (1988). Primary dermal irritation study in the rabbit (Test material: MRD-87-113).
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Acute (less than or equal to 24 hr) 4 hours dermal irritaiton study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980183

Domain	Metric		Rating	Comments
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was appropriate (3/sex).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive to endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rabbits.
	Metric 19:	Blinding of Assessors	Medium	Blinding was not reported; however, outputs were either not subjective (death, weights) or not required per OECD guidelines.
	Metric 20:	Negative Control Response	N/A	The negative control area was not included.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Animals were exposed to the neat test substance and then, 7 days later, exposed to the moistened test substance in a different area. This is a deviation from OECD guidelines. There is uncertainty whether the reaction to the second application of the moistened test substance may have been the result of a sensitization and not irritation. However, this is not a confounding factor for this outcome of interest.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis is not possible since there is not a control group.
	Metric 24:	Reporting of Data	High	Data are fully reported for each animal.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	IIT Research Institute, (1995). Pulmonary sensory irritation study (RD50) of phthalic anhydride dust in the rat. Final report.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Respiratory rate was not affected
Duration:	Acute (less than or equal to 24 hr) at least 10 minutes
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6301186; Linked HERO ID(s): 6301186, 6301188

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No. 85-44-9) and was received in the form of white flakes.
	Metric 2:	Test Substance Source	High	The test substance was supplied by Stepan Company in Northfield, IL. It was identified with lot number 2282. Characterization of the test substance was the responsibility of the supplier.
	Metric 3:	Test Substance Purity	High	The test substance purity was reported as 99.9% by the supplier, and analysis of the purity was the responsibility of the supplier.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Pre-exposure measurements of each animal allowed each animal to serve as it's own control. Animals were acclimated to the exposure chambers for 0.5-1 hour on the day before exposure to the test substance occurred. Respiratory rates were measured directly before the exposures occurred on the day of the experiment.
	Metric 5:	Positive Controls	N/A	Positive controls are not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	The study reported that 4 rats were randomly selected via computer from the group of healthy quarantined rats to include in the study as the exposure group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	The test substance was stored at room temperature (approximately 22 deg. C) in the original container (5-gallon plastic pail). The method of test atmosphere generation as a dust was detailed in the methods and cited to another report ("Test Atmosphere Development of Phthalic Anhydride Dust and Vapor (LO8552)"), which was attached at the end of this study.
	Metric 8:	Consistency of Exposure Administration	Medium	The sample duration and volume for sample number 1 was longer and larger than the other 3 samples for the analytical chamber concentration measurements. Phthalic anhydride and phthalic acid were monitored in the test atmospheres, and were analyzed at different concentrations in each sample. Phthalic anhydride vapors and phthalic acid were detected in the test atmospheres, but were reported to be at negligible concentrations (0.05% and 0.93%, respectively).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal and gravimetric chamber concentrations were reported (42 and 0.574 mg/L, respectively). The average particle size of the dust was 4.95 +/- 1.87 um (MMAD). The mean analytical concentration of phthalic anhydride was 0.486 mg/L. The full range of concentrations within the treatment group was not provided.

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Study Citation:	IIT Research Institute, (1995). Pulmonary sensory irritation study (RD50) of phthalic anhydride dust in the rat. Final report.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Respiratory rate was not affected
Duration:	Acute (less than or equal to 24 hr) at least 10 minutes
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6301186; Linked HERO ID(s): 6301186, 6301188

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Frequency and Duration	Low	Rats were exposed to the test substance for at least 10 minutes, and the average respiratory rate during exposure was calculated over 3 minutes after respiratory rates stabilized. No effects to respiratory rate were observed, so it is not clear if the exposure duration was sufficient to be able to detect an effect.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	The authors reported their target was to achieve the maximum obtainable chamber concentration of phthalic anhydride dust. However, this was not high enough to produce an effect on respiratory rate. Additionally, only one dose group was used. The objective of the study was to determine the concentration that produced a 50% decrease in respiratory rate.
	Metric 12:	Exposure Route and Method	Medium	The number of air changes was not provided, but airflow through the chamber was maintained at 122 L/minute during the exposure period. Head-only exposure chambers were used.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Male Sprague-Dawley (Crl:CDBR) rats were used in this study and were purchased from Charles River Laboratories in Portage, MI with starting bodyweights of 246-272 g. Bodyweights of animals included in the study ranged from 290-305 g. Animals were purchased at 8 weeks of age.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	During the quarantine period prior to exposure, animals were housed individually, provided with food and water ad libitum (except during exposure), with the temperature at 22.8 deg. C and 48% humidity. Animals were maintained on a 12-hour light-dark cycle.
	Metric 15:	Number of Animals per Group	Low	4 animals were used in the experiment (one exposure group).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	The method of respiratory rate measurement was described in the methods and was performed using ventilated plexiglass plethysmograph. Respiratory rates were calculated in real time at 20-second intervals. Respiratory rate was measured in the absence of any other endpoint evaluations (gross pathology or histopathology of the lungs, etc.).
	Metric 17:	Consistency of Outcome Assessment	Medium	The outcome was assessed for each individual animal and individual respiratory rates were established for the pre-, during, and post-exposure periods, but minor details of the outcome assessment protocol execution were not provided (exact timing of each reading, etc.).
	Metric 18:	Sampling Adequacy	High	All 4/4 of the animals were included in the final evaluation of respiratory rate and respiratory rates prior to, during, and after the exposure were provided for each of these animals in Table 2.
	Metric 19:	Blinding of Assessors	N/A	Blinding of assessors is not required for respiratory rate assessment.
	Metric 20:	Negative Control Response	High	The pre-exposure respiratory rates for each animal were used as the baseline/negative control and were provided in Table 2.

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Study Citation:	IIT Research Institute, (1995). Pulmonary sensory irritation study (RD50) of phthalic anhydride dust in the rat. Final report.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Respiratory rate was not affected
Duration:	Acute (less than or equal to 24 hr) at least 10 minutes
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6301186; Linked HERO ID(s): 6301186, 6301188

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	Respiratory rates were provided for each animal. A range of bodyweights were provided at the time animals were obtained and at the start of the study. No other endpoints were evaluated in this study. The study did not report all information to determine confounding, but the reported information did not identify any differences.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure.
	Metric 23: Data Presentation and Analysis	High	Means +/- SD of the respiratory rates were provided. Sufficient data were provided to conduct an independent statistical analysis.
	Metric 24: Reporting of Data	High	The results for mean respiratory rates prior to, during, and after exposure are provided in Table 2. The values are an average of 6-11, 20-second interval readings.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	IIT Research Institute, (1995). Pulmonary sensory irritation study (RD50) of phthalic anhydride vapor in the rat (Final report).
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Respiratory rate was not significantly affected following exposure.
Duration:	Acute (less than or equal to 24 hr) at least 10 minutes
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6301188; Linked HERO ID(s): 6301186, 6301188

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No. 85-44-9) and was received in the form of white flakes.
	Metric 2:	Test Substance Source	High	The test substance was supplied by Stepan Company in Northfield, IL. It was identified with lot number 2282. Characterization of the test substance was the responsibility of the supplier.
	Metric 3:	Test Substance Purity	High	The test substance purity was reported as 99.9% by the supplier, and analysis of the purity was the responsibility of the supplier.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Pre-exposure measurements of each animal allowed each animal to serve as it's own control. Animals were acclimated to the exposure chambers for 0.5-1 hour on the day before exposure to the test substance occurred. Respiratory rates were measured directly before the exposures occurred on the day of the experiment.
	Metric 5:	Positive Controls	N/A	Positive controls are not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	The study reported that 4 rats were randomly selected via computer from the group of healthy quarantined rats to include in the study as the exposure group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	The test substance was stored at room temperature (approximately 22 deg. C) in the original container (5-gallon plastic pail). The method of test atmosphere generation as a vapor was detailed in the methods and cited to another report ("Test Atmosphere Development of Phthalic Anhydride Dust and Vapor (LO8552)"), which was attached at the end of this study.
	Metric 8:	Consistency of Exposure Administration	Medium	The sample duration and volume was similar in both samples collected, but the sample duration was longer than the exposure period used. Phthalic anhydride concentrations were similar but not the same in the two samples collected for measuring the test atmosphere concentrations of phthalic anhydride. The study authors reported no aerosols were detected, so particle size analysis was not performed. Phthalic acid was monitored for, but not detected in the test atmospheres.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal and analytical chamber concentrations were reported (0.03 and 0.011 mg/L, respectively). The maximum obtainable chamber concentration of phthalic anhydride vapor, without any aerosol component, was the target concentration using environmental chamber conditions that could also be used in longer-term studies. The mean analytical concentrations +/- SD for 2 samples were provided, but the range of concentrations within the treatment group was not provided.

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Study Citation:	IIT Research Institute, (1995). Pulmonary sensory irritation study (RD50) of phthalic anhydride vapor in the rat (Final report).
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Respiratory rate was not significantly affected following exposure.
Duration:	Acute (less than or equal to 24 hr) at least 10 minutes
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6301188; Linked HERO ID(s): 6301186, 6301188

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Frequency and Duration	Low	Rats were exposed to the test substance for at least 10 minutes, and the average respiratory rate during exposure was calculated over 3 minutes after respiratory rates stabilized. No effects to respiratory rate were observed, so it is not clear if the exposure duration was sufficient to be able to detect an effect.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	The authors reported their target was to achieve the maximum obtainable chamber concentration of phthalic anhydride vapor, in the absence of aerosol. However, this was not high enough to produce an effect on respiratory rate. Additionally, only one dose group was used. The objective of the study was to determine the concentration that produced a 50% decrease in respiratory rate.
	Metric 12:	Exposure Route and Method	Medium	The number of air changes was not provided, but airflow through the chamber was maintained at 156 L/minute during the exposure period. Head-only exposure chambers were used.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Male Sprague-Dawley (Crl:CDBR) rats were used in this study and were purchased from Charles River Laboratories in Portage, MI with starting bodyweights of 10% of animals between 149-170 g. Bodyweights of animals included in the study ranged from 210-243 g. Animals were purchased at 6 weeks of age.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	During the quarantine period prior to exposure, animals were housed individually, provided with food and water ad libitum (except during exposure), with the temperature at 23.9 deg. C and 58% humidity. Animals were maintained on a 12-hour light-dark cycle.
	Metric 15:	Number of Animals per Group	Low	4 animals were used in the experiment (one exposure group). The baseline respiratory rate was not reached for one animal, so only 3 animals were included in the final analysis.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	The method of respiratory rate measurement was described in the methods and was performed using ventilated plexiglass plethysmograph. Respiratory rates were calculated in real time at 10-second intervals. Respiratory rate was measured in the absence of any other endpoint evaluations (gross pathology or histopathology of the lungs, etc.).
	Metric 17:	Consistency of Outcome Assessment	Medium	The outcome was assessed for each individual animal and individual respiratory rates were established for the pre-, during, and post-exposure periods, but minor details of the outcome assessment protocol execution were not provided (exact timing of each reading, etc.).
	Metric 18:	Sampling Adequacy	Medium	3/4 of the animals were included in the final evaluation of respiratory rate and respiratory rates prior to, during, and after the exposure were provided for each of these 3 animals in Table 2.
	Metric 19:	Blinding of Assessors	N/A	Blinding of assessors is not required for respiratory rate assessment.

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Study Citation:	IIT Research Institute, (1995). Pulmonary sensory irritation study (RD50) of phthalic anhydride vapor in the rat (Final report).
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Respiratory rate was not significantly affected following exposure.
Duration:	Acute (less than or equal to 24 hr) at least 10 minutes
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6301188; Linked HERO ID(s): 6301186, 6301188

Domain	Metric		Rating	Comments
	Metric 20:	Negative Control Response	Medium	The pre-exposure respiratory rates for each animal were used as the baseline/negative control. The pre-exposure respiratory rate was not stable for one animal, and it was therefore excluded; no data was reported for this animal so it is not clear what this animal's respiratory rate was during the pre-exposure measurement. The authors did not further describe the reasons as to why the baseline was not stable prior to exposure.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	The unstable respiratory rate of one animal was not provided, so it is not clear if this would have been a confounding factor. This animal was not included in the final analysis. A range of bodyweights were provided at the time animals were obtained and at the start of the study. No other endpoints were evaluated in this study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	High	Means +/- SD of the respiratory rates were provided. Sufficient data were provided to conduct an independent statistical analysis.
	Metric 24:	Reporting of Data	High	The results for mean respiratory rates prior to, during, and after exposure are provided in Table 2. The values are an average of 7-19, 10-second interval readings.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	IT,, Bayer (1979). Examination for skin and mucous membrane tolerance (Test sample: Phthalic anhydride).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Skin irritation, eye irritation
Duration:	Acute (less than or equal to 24 hr) Rabbit eye irritation acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980172

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by name as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	A separate negative or vehicle control group is not required for eye irritation studies. For eye irritation studies, typically the chemical is applied to one eye, and the untreated eye serves as the control. Insufficient information was provided to determine whether one eye was left untreated.
	Metric 5:	Positive Controls	N/A	A positive control is not required for acute eye irritation studies.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	No details on the preparation and storage of the test substance were provided. The test substance was applied once, so only a single preparation was needed.
	Metric 8:	Consistency of Exposure Administration	Low	Insufficient details were provided to determine whether exposure administration was consistent. The volume of test chemical applied was not reported.
	Metric 9:	Reporting of Doses/Concentrations	Uninformative	Neither the exposure concentration or volume applied was reported.
	Metric 10:	Exposure Frequency and Duration	High	The test substance was applied once, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	The study tested a single exposure group. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	Animals were dosed via application into the conjunctival sac, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The study used male and female New Zealand White rabbits. Initial body weights were reported. Animal age and source were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Test animals were housed individually. No other husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The study used 2 animals, which is adequate for an acute eye irritation study.
Domain 5: Outcome Assessment				

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Study Citation:	IT,, Bayer (1979). Examination for skin and mucous membrane tolerance (Test sample: Phthalic anhydride).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Skin irritation, eye irritation
Duration:	Acute (less than or equal to 24 hr) Rabbit eye irritation acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980172

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Low	Limited details are provided, but animals were observed for irritation for 7 days; if no irritation is observed, this is appropriate. However, the frequency of observations and the method of grading reactions were not specified, which is a significant deficiency.
	Metric 17:	Consistency of Outcome Assessment	Low	Insufficient data are provided to determine whether there was consistency of outcome assessment.
	Metric 18:	Sampling Adequacy	Low	Details regarding the sampling of outcomes (e.g. frequency of observation) were not reported.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not mentioned in OECD TG 405; however, it is mentioned that the grading of ocular responses is necessarily subjective and that persons conducting the examinations should be adequately trained. Blinding or qualifications were not mentioned in the study report.
	Metric 20:	Negative Control Response	N/A	The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment. While the study did not report body weights or food or water intake, these are not a requirement for an eye irritation study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis of eye irritation was not necessary.
	Metric 24:	Reporting of Data	Low	Irritation scores were not reported. The response was qualitatively described.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	IT,, Bayer (1979). Examination for skin and mucous membrane tolerance (Test sample: Phthalic anhydride).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Skin irritation, eye irritation
Duration:	Acute (less than or equal to 24 hr) Rabbit dermal acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980172

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by name as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A negative or vehicle control is not required for acute dermal studies. Typically, unexposed areas of skin serve as a negative control
	Metric 5:	Positive Controls	N/A	Positive controls are not required for dermal irritation studies.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	No details on the preparation and storage of the test substance were provided. It is unclear if the test substance was moistened prior to application. The test substance was applied once, so only a single preparation was needed.
	Metric 8:	Consistency of Exposure Administration	Low	Insufficient details were provided to determine whether exposure administration was consistent across groups. Animals were dermally exposed under occlusion for 24 hours, after which the exposed areas were cleaned. Site preparation details and surface area of application were not reported. It is unclear whether the test substance was moistened and if there was good dermal contact.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The amount of test chemical applied to each test animal was reported. Animals received a dose of 500 mg/animal. An initial animal body weight range was provided, so a mg/kg dose can be estimated.
	Metric 10:	Exposure Frequency and Duration	High	The test substance was applied once, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	The study tested a single exposure group. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	Medium	Animals were dosed by application of the test chemical to the ear under occlusion. The dorsal area is preferred for skin irritation studies. It is assumed that the skin was intact, but this was not explicitly stated. OECD guidelines recommend a semi-occlusive, rather than an occlusive dressing.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The study used male and female New Zealand White rabbits. Initial body weights were reported. Animal age and source were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Test animals were housed individually. No other husbandry conditions were reported.

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Study Citation:	IT,, Bayer (1979). Examination for skin and mucous membrane tolerance (Test sample: Phthalic anhydride).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Skin irritation, eye irritation
Duration:	Acute (less than or equal to 24 hr) Rabbit dermal acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980172

Domain		Metric	Rating	Comments
	Metric 15:	Number of Animals per Group	Medium	The study used 2 animals, which is adequate for a dermal irritation study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	Limited details are provided, but animals were observed for irritation for 7 days. Typically, animals are observed for 14 days to assess reversibility; however, if no irritation is observed, 7 days is appropriate. The method of scoring skin irritation was not provided, which is a significant deficiency.
	Metric 17:	Consistency of Outcome Assessment	Low	Insufficient data are provided to determine whether there was consistency of outcome assessment.
	Metric 18:	Sampling Adequacy	Low	Details regarding the sampling of outcomes (e.g. frequency of observation) were not reported.
	Metric 19:	Blinding of Assessors	N/A	Skin examinations may be subjective, but blinding is not specified as being required in OECD TG for acute dermal irritation studies.
	Metric 20:	Negative Control Response	N/A	The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment. While the study did not report body weights or food or water intake, these are not a requirement for a dermal study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis of skin irritation was not necessary.
	Metric 24:	Reporting of Data	Low	Irritation scores were not reported. The response was qualitatively described.

Additional Comments: None

Overall Quality Determination — Low

Study Citation:	Jha, A. M., Singh, A. C., Bharti, M. (1998). Germ cell mutagenicity of phthalic acid in mice. Mutation Research 422(2):207-212.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: -Sperm head abnormality-Dominant lethal mutation
Duration:	Acute (less than or equal to 24 hr) Acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1336719

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	The source of test substance was not reported. Lot/Batch number was not reported.
	Metric 3:	Test Substance Purity	High	The purity of the test substance was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A negative control group was included and appropriate.
	Metric 5:	Positive Controls	N/A	Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Preparation and storage were partially described. Given the chemical properties of the test substance, this is unlikely to have a substantial effect on the results.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was consistent across the study groups
	Metric 9:	Reporting of Doses/Concentrations	High	Exposure was consistent across the study groups
	Metric 10:	Exposure Frequency and Duration	High	Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	A NOAEL was not determined.
	Metric 12:	Exposure Route and Method	High	The exposure route was appropriate.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Not all husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals treated per group was appropriate
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Methodology was appropriate for outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Medium	Details regarding sampling of outcomes were not reported.
	Metric 19:	Blinding of Assessors	N/A	Not applicable.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.

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Study Citation:	Jha, A. M., Singh, A. C., Bharti, M. (1998). Germ cell mutagenicity of phthalic acid in mice. Mutation Research 422(2):207-212.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: -Sperm head abnormality-Dominant lethal mutation
Duration:	Acute (less than or equal to 24 hr) Acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1336719

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Jha, A. M., Singh, A. C., Bharti, M. (1998). Germ cell mutagenicity of phthalic acid in mice. Mutation Research 422(2):207-212.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) Acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1336719

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	The source of test substance was not reported. Lot/Batch number was not reported.
	Metric 3:	Test Substance Purity	High	The purity of the test substance was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A negative control was not applicable for lethality study.
	Metric 5:	Positive Controls	N/A	Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Preparation and storage were partially described. Given the chemical properties of the test substance, this is unlikely to have a substantial effect on the results.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was consistent across the study groups
	Metric 9:	Reporting of Doses/Concentrations	High	Exposure was consistent across the study groups
	Metric 10:	Exposure Frequency and Duration	High	Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups was appropriate
	Metric 12:	Exposure Route and Method	High	The exposure route was appropriate.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Not all husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals treated per group was appropriate
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Methodology was appropriate for outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not necessary for this study
	Metric 20:	Negative Control Response	N/A	Not needed for lethality study.
Domain 6: Confounding / Variable Control				

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Study Citation:	Jha, A. M., Singh, A. C., Bharti, M. (1998). Germ cell mutagenicity of phthalic acid in mice. Mutation Research 422(2):207-212.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) Acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1336719

Domain	Metric		Rating	Comments
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	MB Research Laboratories Inc, (1979). Test for eye irritation in rabbits (redacted).
Health Outcome(s):	Irritation (Eye)
Reported Health Effect(s):	Irritation (Eye): Eye (cornea, iris, conjunctiva irritation)
Duration:	Acute (less than or equal to 24 hr) Acute eye irritiation
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980179

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	The test substance was identified as phthalic anhydride residue in a companion document that was submitted with the TSCA 8d submission (HERO 13028219). No further information was provided in either document indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A negative or vehicle control group is not required for eye irritation studies. The untreated eye of each study animal served as a negative control.
	Metric 5:	Positive Controls	N/A	Positive controls are not required for eye irritation studies.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into the test group was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	No details on preparation and storage of the test substance were provided. The test substance was applied once, so only a single preparation was needed.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal amount of test substance applied was reported. Animals were administered a single nominal dose of 0.1 g/animal. No information on body weight was reported, so a mg/kg dose cannot be estimated.
	Metric 10:	Exposure Frequency and Duration	High	The test substance was applied once, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	The study tested a single exposure group. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	Animals were dosed via instillation into the conjunctival sac, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male and female New Zealand White rabbits. The source and age of the test animals were reported. Initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Test animals were housed individually. Some husbandry conditions (e.g. temperature, diet, and water availability) were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 6 test animals.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for eye irritation in rabbits (redacted).
Health Outcome(s):	Irritation (Eye)
Reported Health Effect(s):	Irritation (Eye): Eye (cornea, iris, conjunctiva irritation)
Duration:	Acute (less than or equal to 24 hr) Acute eye irritiation
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980179

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	The study does not indicate whether eyes were washed after installation or if the solid test material remained in the eye after the first hours. OECD 405 guidelines do not recommend washing the eye; however they do recommend removing the solid test material from the eye after 1 hour if it was not removed by physiological mechanisms already. The cornea, iris, and conjunctiva were examined after 1 and 4 hr, as well as after 1, 2, 3, 4 and 7 days. Animals with positive irritation scores after 7 days were examined again on day 10. Animals with positive irritation scores on day 10 were examined on day 14.
	Metric 17:	Consistency of Outcome Assessment	High	Eye irritation was assessed in all test animals according to the same protocol and time intervals.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were examined for eye irritation.
	Metric 19:	Blinding of Assessors	Low	Blinding is not mentioned in OECD TG 405; however, it is mentioned that the grading of ocular responses is necessarily subjective and that persons conducting the examinations should be adequately trained. Blinding or qualifications were not mentioned in the study report.
	Metric 20:	Negative Control Response	N/A	The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment. While the study did not report body weights or food or water intake, these are not a requirement for an eye irritation study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment. The health of animal eyes was confirmed prior to the start of the study.
	Metric 23:	Data Presentation and Analysis	N/A	An irritation score was calculated. Statistical analysis is not required for this study type.
	Metric 24:	Reporting of Data	High	Irritation scores for each test animal were reported.

Additional Comments: This study was deemed unacceptable due to the uncertainty of the test substance.

Overall Quality Determination — Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for acute dermal toxicity/LD 50 in rabbits.
Health Outcome(s):	Mortality; Irritation (Skin); Nutritional/Metabolic; Neurological/Behavioral; Gastrointestinal;
Reported Health Effect(s):	Mortality: Mortality; Irritation (Skin): Skin irritation; Nutritional/Metabolic: Body weight; Neurological/Behavioral: Clinical signs; Gastrointestinal: Clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute dermal toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980180

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	All Outcomes: The test substance was identified as phthalic anhydride residue in a companion document that was submitted with the TSCA 8d submission (HERO 13028219). No further information was provided in either document indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A negative or vehicle control is not required for dermal toxicity studies. Typically, unexposed areas of skin serve as a negative control.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive controls are not required for dermal toxicity studies.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: No information on the methods of allocation of animals into the test group was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: The test chemical was dissolved in Mazola oil. No details on storage of the test substance were provided. The test substance was applied once, so only a single preparation was needed.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Details of exposure administration were reported, and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Mortality: Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.; Irritation (Skin): Doses were clearly reported. Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.; Nutritional/Metabolic: Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.; Neurological/Behavioral: Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.; Gastrointestinal: Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for acute dermal toxicity/LD 50 in rabbits.
Health Outcome(s):	Mortality; Irritation (Skin); Nutritional/Metabolic; Neurological/Behavioral; Gastrointestinal;
Reported Health Effect(s):	Mortality: Mortality; Irritation (Skin): Skin irritation; Nutritional/Metabolic: Body weight; Neurological/Behavioral: Clinical signs; Gastrointestinal: Clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute dermal toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980180

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The test substance was applied once, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	All Outcomes: The study tested a single exposure group which was justified by the study authors. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	Uninformative	All Outcomes: The study clipped the fur from the abdomen of the animals and then made abrasions in the skin. The abrasions ran the entire length of the exposure site. They were scratched into the stratum corneum, but did not reach the derma or produce bleeding. The OECD guidelines state that "Care must be taken to avoid abrading the skin, which could alter its permeability." The unabraded dorsal area is preferred.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The study used male and female New Zealand White rabbits. The source, sex, age, and initial body weights of the test animals were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Test animals were housed individually. Some husbandry conditions (e.g. temperature, diet, and water availability) were reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals was adequate. The study used 4 test animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Mortality: The skin was cleaned after 24 hours and animals were observed at least once per day for 14 days for mortality, signs of irritation, and signs of toxicity.; Irritation (Skin): The skin was cleaned after 24 hours and animals were observed at least once per day for 14 days for mortality, signs of irritation, and signs of toxicity.; Nutritional/Metabolic: The skin was cleaned after 24 hours and animals were observed at least once per day for 14 days for mortality, signs of irritation, and signs of toxicity. Body weights were taken at day 0 and 14.; Neurological/Behavioral: The skin was cleaned after 24 hours and animals were observed at least once per day for 14 days for mortality, signs of irritation, and signs of toxicity.; Gastrointestinal: The skin was cleaned after 24 hours and animals were observed at least once per day for 14 days for mortality, signs of irritation, and signs of toxicity.
	Metric 17:	Consistency of Outcome Assessment	High	Mortality: Mortality was assessed in all test animals according to the same protocol and time intervals.; Irritation (Skin): Test animals were observed 1, 3, 7, 10, and 14 days after dosing for signs of skin irritation. Dermal reactions were scored by the Draize scoring system.; Nutritional/Metabolic: Test animal body weights were examined consistently across groups.; Neurological/Behavioral: Signs of toxicity were assessed in all test animals according to the same protocol and time intervals.; Gastrointestinal: Signs of toxicity were assessed in all test animals according to the same protocol and time intervals.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for acute dermal toxicity/LD 50 in rabbits.
Health Outcome(s):	Mortality; Irritation (Skin); Nutritional/Metabolic; Neurological/Behavioral; Gastrointestinal;
Reported Health Effect(s):	Mortality: Mortality; Irritation (Skin): Skin irritation; Nutritional/Metabolic: Body weight; Neurological/Behavioral: Clinical signs; Gastrointestinal: Clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute dermal toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980180

Domain	Metric		Rating	Comments
	Metric 18:	Sampling Adequacy	High	Mortality: All of the animals used in the study were observed for mortality at 2 and 4 hours post dosing and daily for 14 days.; Irritation (Skin): All of the animals used in the study were examined for dermal reactions 1, 3, 7, 10, and 14 days after dosing.; Nutritional/Metabolic: All of the animals used in the study were weighed before dosing and at the conclusion of the study period (14 days after dosing).; Neurological/Behavioral: Sampling was adequate for the outcome of clinical signs. All of the animals used in the study were observed for signs of toxicity at 2 and 4 hours post dosing and daily for 14 days.; Gastrointestinal: Sampling was adequate for the outcome of clinical signs. All of the animals used in the study were observed for signs of toxicity at 2 and 4 hours post dosing and daily for 14 days.
	Metric 19:	Blinding of Assessors	N/A	Mortality: Blinding is not required for this endpoint.; Irritation (Skin): Blinding is not required for most of the outcomes of interest. Skin examinations may be subjective, but blinding is not specified as being required in OECD TG for acute dermal irritation studies.; Nutritional/Metabolic: Blinding is not required for this endpoint.; Neurological/Behavioral: The study did not report whether assessors were blinded to treatment group but was not necessary for assessing clinical signs.; Gastrointestinal: The study did not report whether assessors were blinded to treatment group but was not necessary for assessing clinical signs.
	Metric 20:	Negative Control Response	N/A	All Outcomes: The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment. While the study did not report food or water intake, these are not a requirement for an acute dermal toxicity study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment. The health of animal was confirmed prior to the start of the study.
	Metric 23:	Data Presentation and Analysis	N/A	Mortality: Statistical analysis was not necessary.; Irritation (Skin): Statistical analysis of skin irritation was not necessary.; Nutritional/Metabolic: Statistical analysis is not possible (no control group); Neurological/Behavioral: Statistical analysis of clinical signs was not necessary.; Gastrointestinal: Statistical analysis of clinical signs was not necessary.
	Metric 24:	Reporting of Data	High	Mortality: Mortality data were summarized for each sex.; Irritation (Skin): Skin irritation was described for each individual test animal.; Nutritional/Metabolic: Body weights were presented for each individual test animal.; Neurological/Behavioral: Clinical signs were described for each individual test animal.; Gastrointestinal: Clinical signs were described for each individual test animal.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for acute dermal toxicity/LD 50 in rabbits.
Health Outcome(s):	Mortality; Irritation (Skin); Nutritional/Metabolic; Neurological/Behavioral; Gastrointestinal;
Reported Health Effect(s):	Mortality: Mortality; Irritation (Skin): Skin irritation; Nutritional/Metabolic: Body weight; Neurological/Behavioral: Clinical signs; Gastrointestinal: Clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute dermal toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980180

Domain	Metric	Rating	Comments

Additional Comments: The study is deemed unacceptable due to uncertainty with the test substance and application of the test substance to abraded skin.

Overall Quality Determination Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for acute dermal toxicity/LD 50 in rabbits.
Health Outcome(s):	Cardiovascular; Reproductive/Developmental;
Reported Health Effect(s):	Cardiovascular: Gross necropsy; Reproductive/Developmental: Gross necropsy;
Duration:	Acute (less than or equal to 24 hr) Acute dermal toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980180

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	All Outcomes: The test substance was identified as phthalic anhydride residue in a companion document that was submitted with the TSCA 8d submission (HERO 13028219). No further information was provided in either document indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A negative or vehicle control is not required for dermal toxicity studies. Typically, unexposed areas of skin serve as a negative control.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive controls are not required for dermal toxicity studies.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: No information on the methods of allocation of animals into the test group was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: The test chemical was dissolved in Mazola oil. No details on storage of the test substance were provided. The test substance was applied once, so only a single preparation was needed.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Details of exposure administration were reported, and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The test substance was applied once, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	All Outcomes: The study tested a single exposure group which was justified by the study authors. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	Uninformative	All Outcomes: The study clipped the fur from the abdomen of the animals and then made abrasions in the skin. The abrasions ran the entire length of the exposure site. They were scratched into the stratum corneum, but did not reach the derma or produce bleeding. The OECD guidelines state that "Care must be taken to avoid abrading the skin, which could alter its permeability." The unabraded dorsal area is preferred.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The study used male and female New Zealand White rabbits. The source, sex, age, and initial body weights of the test animals were reported.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for acute dermal toxicity/LD 50 in rabbits.
Health Outcome(s):	Cardiovascular; Reproductive/Developmental;
Reported Health Effect(s):	Cardiovascular: Gross necropsy; Reproductive/Developmental: Gross necropsy;
Duration:	Acute (less than or equal to 24 hr) Acute dermal toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980180

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Test animals were housed individually. Some husbandry conditions (e.g. temperature, diet, and water availability) were reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals was adequate. The study used 4 test animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Cardiovascular: Gross necropsy was performed at the time of sacrifice.; Reproductive/Developmental: Gross necropsy was performed.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Gross necropsy was performed on all test animals upon sacrifice at the end of the study period.
	Metric 18:	Sampling Adequacy	High	All Outcomes: All of the animals used in the study were examined by gross necropsy.
	Metric 19:	Blinding of Assessors	Medium	All Outcomes: The study did not report whether assessors were blinded to treatment group for the gross necropsy, and this is not likely to have a substantial impact on results.
	Metric 20:	Negative Control Response	N/A	All Outcomes: The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment. While the study did not report food or water intake, these are not a requirement for an acute dermal toxicity study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment. The health of animal was confirmed prior to the start of the study.
	Metric 23:	Data Presentation and Analysis	N/A	All Outcomes: Statistical analysis of gross necropsy findings was not necessary.
	Metric 24:	Reporting of Data	High	All Outcomes: Gross necropsy findings were described for each individual test animal.

Additional Comments: The study is deemed unacceptable due to uncertainty with the test substance and application of the test substance to abraded skin.

Overall Quality Determination — Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Nutritional/Metabolic; Mortality-related clinical signs (Mortality-related clinical signs);
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; Mortality-related clinical signs (Mortality-related clinical signs): Mortality-related clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	All Outcomes: The test substance was identified as phthalic anhydride residue in a companion document that was submitted with the TSCA 8d submission (HERO 13028219). No further information was provided in either document indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A negative or vehicle control is not required for acute oral lethality studies.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive controls are not required for acute oral lethality studies.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: No information on the methods of allocation of animals into the test group was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: The test chemical was dissolved in Mazola oil (50% w/v). No details on storage of the test substance were provided. Animals were administered one dose, so frequency details are not needed.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: Details of exposure administration were reported. Gavage volume varied among exposure groups from 2 to 14 ml/kg but were consistent within each dose group. The gavage volume in the highest dose group exceeded 10 ml/kg.
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses were clearly reported. Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Animals were administered a single dose, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: The number of exposure groups and dose spacing sufficiently covered the full range of responses.
	Metric 12:	Exposure Route and Method	High	All Outcomes: Animals were dosed via gavage, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				

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Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Nutritional/Metabolic; Mortality-related clinical signs (Mortality-related clinical signs);
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; Mortality-related clinical signs (Mortality-related clinical signs): Mortality-related clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The study used male Wistar rats. The source, sex, age, and initial body weights of the test animals were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Test animals were housed individually. Some husbandry conditions (e.g. temperature, diet, and water availability) were reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals was adequate. The study used 5 test animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: Test animals were observed 1, 2, 4, and 6 hours after dosing and daily for 14 days for mortality and signs of toxicity.
	Metric 17:	Consistency of Outcome Assessment	High	Nutritional/Metabolic: Test animal body weights were examined consistently across groups.; Mortality-related clinical signs (Mortality-related clinical signs): Signs of toxicity were assessed in all test animals according to the same protocol and time intervals.
	Metric 18:	Sampling Adequacy	High	Nutritional/Metabolic: All of the animals used in the study were weighed before dosing and at the conclusion of the study period (14 days after dosing).; Mortality-related clinical signs (Mortality-related clinical signs): Sampling was adequate for the outcome of clinical signs. All of the animals used in the study were observed for signs of toxicity at 1, 2, 4, and 6 hours after dosing and daily for 14 days.
	Metric 19:	Blinding of Assessors	N/A	Nutritional/Metabolic: Blinding is not required for this endpoint.; Mortality-related clinical signs (Mortality-related clinical signs): The study did not report whether assessors were blinded to treatment group for evaluating clinical signs, however this is not required.
	Metric 20:	Negative Control Response	N/A	All Outcomes: The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: There were marked differences (≥10%) in initial body weights between exposure groups. While the study did not report food or water intake, these are not a requirement for a gavage study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment. The health of animal was confirmed prior to the start of the study.
	Metric 23:	Data Presentation and Analysis	N/A	Nutritional/Metabolic: Statistical analysis was not applicable, as there was no negative control group.; Mortality-related clinical signs (Mortality-related clinical signs): Statistical analysis of clinical signs was not necessary.
	Metric 24:	Reporting of Data	High	Nutritional/Metabolic: Body weights were presented for each individual test animal.; Mortality-related clinical signs (Mortality-related clinical signs): Clinical signs were described for each individual test animal.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Nutritional/Metabolic; Mortality-related clinical signs (Mortality-related clinical signs);
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; Mortality-related clinical signs (Mortality-related clinical signs): Mortality-related clinical signs;
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric	Rating	Comments

Additional Comments: This study was deemed unacceptable due to the lack of information on the test substance. It was identified only as phthalic anhydride residue.

Overall Quality Determination Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	The test substance was identified as phthalic anhydride residue in a companion document that was submitted with the TSCA 8d submission (HERO 13028219). No further information was provided in either document indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A negative or vehicle control is not required for acute oral lethality studies.
	Metric 5:	Positive Controls	N/A	Positive controls are not required for acute oral lethality studies.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into the test group was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test chemical was dissolved in Mazola oil (50% w/v). No details on storage of the test substance were provided. Animals were administered one dose, so frequency details are not needed.
	Metric 8:	Consistency of Exposure Administration	Low	Details of exposure administration were reported. Gavage volume varied among exposure groups from 2 to 14 ml/kg but were consistent within each dose group. The gavage volume in the highest dose group exceeded 10 ml/kg.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Nominal doses were clearly reported. Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	Animals were administered a single dose, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose spacing sufficiently covered the full range of responses.
	Metric 12:	Exposure Route and Method	High	Animals were dosed via gavage, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The study used male Wistar rats. The source, sex, age, and initial body weights of the test animals were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Test animals were housed individually. Some husbandry conditions (e.g. temperature, diet, and water availability) were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 5 test animals per group.
Domain 5: Outcome Assessment				

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Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	Test animals were observed 1, 2, 4, and 6 hours after dosing and daily for 14 days for mortality and signs of toxicity.
	Metric 17:	Consistency of Outcome Assessment	High	Mortality was assessed in all test animals according to the same protocol and time intervals.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were observed for mortality at 1, 2, 4, and 6 hours after dosing and daily for 14 days.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	N/A	The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	There were marked differences (≥10%) in initial body weights between exposure groups. While the study did not report food or water intake, these are not a requirement for a gavage study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment. The health of animal was confirmed prior to the start of the study.
	Metric 23:	Data Presentation and Analysis	High	The statistical method used to calculate LC50 was reported and appropriate.
	Metric 24:	Reporting of Data	High	Mortality data were presented for each individual test animal, as well as by study group.

Additional Comments: This study was deemed unacceptable due to the lack of information on the test substance. It was identified only as phthalic anhydride residue.

Overall Quality Determination — Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Reproductive/Developmental; Gastrointestinal; Lung/Respiratory; Cardiovascular;
Reported Health Effect(s):	Reproductive/Developmental: Gross necropsy; Gastrointestinal: Gross necropsy; Lung/Respiratory: Gross necropsy; Cardiovascular: Gross necropsy;
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	All Outcomes: The test substance was identified as phthalic anhydride residue in a companion document that was submitted with the TSCA 8d submission (HERO 13028219). No further information was provided in either document indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A negative or vehicle control is not required for acute oral lethality studies.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive controls are not required for acute oral lethality studies.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: No information on the methods of allocation of animals into the test group was reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: The test chemical was dissolved in Mazola oil (50% w/v). No details on storage of the test substance were provided. Animals were administered one dose, so frequency details are not needed.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: Details of exposure administration were reported. Gavage volume varied among exposure groups from 2 to 14 ml/kg but were consistent within each dose group. The gavage volume in the highest dose group exceeded 10 ml/kg.
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses were clearly reported. Animals were administered a single nominal dose. The concentration in the test solution was not verified; however, given the single dose preparation required, this is unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Animals were administered a single dose, which is appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: The number of exposure groups and dose spacing sufficiently covered the full range of responses.
	Metric 12:	Exposure Route and Method	High	All Outcomes: Animals were dosed via gavage, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The study used male Wistar rats. The source, sex, age, and initial body weights of the test animals were reported.

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Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (October 1979).
Health Outcome(s):	Reproductive/Developmental; Gastrointestinal; Lung/Respiratory; Cardiovascular;
Reported Health Effect(s):	Reproductive/Developmental: Gross necropsy; Gastrointestinal: Gross necropsy; Lung/Respiratory: Gross necropsy; Cardiovascular: Gross necropsy;
Duration:	Acute (less than or equal to 24 hr) Acute oral toxicity
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980186

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Test animals were housed individually. Some husbandry conditions (e.g. temperature, diet, and water availability) were reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals was adequate. The study used 5 test animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: Gross necropsy was performed at time of sacrifice.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Gross necropsy was performed on all test animals upon sacrifice at the end of the study period.
	Metric 18:	Sampling Adequacy	High	All Outcomes: All of the animals used in the study were examined by gross necropsy.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: The study did not report whether assessors were blinded to treatment group for evaluating gross necropsy, however this is not required.
	Metric 20:	Negative Control Response	N/A	All Outcomes: The study did not include negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: There were marked differences (≥10%) in initial body weights between exposure groups. While the study did not report food or water intake, these are not a requirement for a gavage study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment. The health of animal was confirmed prior to the start of the study.
	Metric 23:	Data Presentation and Analysis	N/A	All Outcomes: Statistical analysis of gross necropsy findings was not necessary.
	Metric 24:	Reporting of Data	High	All Outcomes: Gross necropsy findings were described for each individual test animal.

Additional Comments: This study was deemed unacceptable due to the lack of information on the test substance. It was identified only as phthalic anhydride residue.

Overall Quality Determination — Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (July 1979).
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Acute (less than or equal to 24 hr) Single gavage
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980187

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	The test substance was identified as MRD-79-15 in the study and phthalic anhydride residue in a companion document (HERO 13028219). No further information was provided in either document, indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not explicitly reported; but is likely from the sponsor (Exxon).
	Metric 3:	Test Substance Purity	Low	The purity or grade of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A concurrent negative control is not required for this study type.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were selected for the study.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test substance was reported to be a 50% w/v mixture in distilled water and heated; no other details are provided. Storage of the test substance was not reported; however, animals were administered a single gavage, so storage of a prepared test substance was not required.
	Metric 8:	Consistency of Exposure Administration	Medium	The gavage volume varied from 4.2 ml to 5.1 ml. The delivered volume was appropriate (0.2 mg/10g body weight).
	Metric 9:	Reporting of Doses/Concentrations	Medium	A nominal dose (10.0 g/kg) was reported. Body weights were reported.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration and frequency were appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only one dose (10.0 g/kg) was tested, which is appropriate when a limit test is conducted. The dose is higher than the recommended limit dose of 2,000 mg/kg in OECD TG 423.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was appropriate (n=5).

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Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (July 1979).
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Acute (less than or equal to 24 hr) Single gavage
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980187

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all animals.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, outputs were either not subjective (death), or clinical sings and gross necropsy.
	Metric 20:	Negative Control Response	N/A	Negative controls were not included in the study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Gavage volumes differed between groups (ranging from 4.2 to 5.1 ml); this was due to the variation in initial body weights (ranged from 211 to 256 g) in the animals.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not applicable (control group not included).
	Metric 24:	Reporting of Data	High	Data are fully reported with incidence data.

Additional Comments: This study was deemed unacceptable due to the lack of information on the test substance. It was identified only as phthalic anhydride residue.

Overall Quality Determination Uninformative

Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (July 1979).
Health Outcome(s):	Neurological/Behavioral; Lung/Respiratory; Ocular/Sensory; Gastrointestinal;
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs (lethargy, flaccid, ataxia, piloerections) - likely associated with mortality; Lung/Respiratory: Clinical signs (dyspnea); Ocular/Sensory: Clinical signs (chromodacryorrhea, chromorhinorrhea, ptosis); Gastrointestinal: Gross necropsy;
Duration:	Acute (less than or equal to 24 hr) Single gavage
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980187

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	All Outcomes: The test substance was identified as MRD-79-15 in the study and phthalic anhydride residue in a companion document (HERO 13028219). No further information was provided in either document, indicating the composition and/or identity of the test substance.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of the test substance was not explicitly reported; but is likely from the sponsor (Exxon).
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity or grade of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A concurrent negative control is not required for this study type.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: The study did not report how animals were selected for the study.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: The test substance was reported to be a 50% w/v mixture in distilled water and heated; no other details are provided. Storage of the test substance was not reported; however, animals were administered a single gavage, so storage of a prepared test substance was not required.
	Metric 8:	Consistency of Exposure Administration	Medium	All Outcomes: The gavage volume varied from 4.2 ml to 5.1 ml. The delivered volume was appropriate (0.2 mg/10g body weight).
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal dose (10.0 g/kg) was reported. Body weights were reported.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure duration and frequency were appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: Only one dose (10.0 g/kg) was tested, which is appropriate when a limit test is conducted. The dose is higher than the recommended limit dose of 2,000 mg/kg in OECD TG 423.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals/group was appropriate (n=5).
Domain 5: Outcome Assessment				

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Study Citation:	MB Research Laboratories Inc, (1979). Test for oral toxicity in rats (July 1979).
Health Outcome(s):	Neurological/Behavioral; Lung/Respiratory; Ocular/Sensory; Gastrointestinal;
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs (lethargy, flaccid, ataxia, piloerections) - likely associated with mortality; Lung/Respiratory: Clinical signs (dyspnea); Ocular/Sensory: Clinical signs (chromodacryorrhea, chromorhinorrhea, ptosis); Gastrointestinal: Gross necropsy;
Duration:	Acute (less than or equal to 24 hr) Single gavage
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980187

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Low	Neurological/Behavioral: Only clinical signs were assessed.; Lung/Respiratory: Only clinical signs were assessed.; Ocular/Sensory: Only clinical signs were assessed.; Gastrointestinal: Only gross necropsy was assessed.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Sampling was sufficient. The study reported findings for all animals.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, outputs were either not subjective (death), or clinical sings and gross necropsy.
	Metric 20:	Negative Control Response	N/A	Neurological/Behavioral: Negative controls were not included in the study.; Lung/Respiratory: Negative controls were not included in the study; Ocular/Sensory: Negative controls were not included in the study.; Gastrointestinal: Negative controls were not included in the study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Gavage volumes differed between groups (ranging from 4.2 to 5.1 ml); this was due to the variation in initial body weights (ranged from 211 to 256 g) in the animals.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	All Outcomes: Statistical analysis was not applicable (control group not included).
	Metric 24:	Reporting of Data	High	All Outcomes: Data are fully reported with incidence data.

Additional Comments: This study was deemed unacceptable due to the lack of information on the test substance. It was identified only as phthalic anhydride residue.

Overall Quality Determination — Uninformative

Study Citation:	Power, A. E., Mcgaugh, J. L. (2002). Cholinergic activation of the basolateral amygdala regulates unlearned freezing behavior in rats. Behavioural Brain Research 134(1-2):307-315.
Health Outcome(s):	Neurological/Behavioral; Nutritional/Metabolic;
Reported Health Effect(s):	Neurological/Behavioral: unconditioned fear behavior, open field activity, shock context; Nutritional/Metabolic: Body weight gain;
Duration:	Acute (less than or equal to 24 hr) single infusion
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6816161

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	All Outcomes: Phthalic acid, CAS number or structure were not reported.
	Metric 2:	Test Substance Source	Low	All Outcomes: Purchased from Sigma; batch/lot number was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A sham control was used
	Metric 5:	Positive Controls	N/A	All Outcomes: this study type does not require a positive control
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: There is an omission of details that are unlikely to have a substantial impact on results
	Metric 8:	Consistency of Exposure Administration	Medium	All Outcomes: Details of exposure administration are incompletely reported
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Concentration of PA infusion was reported as (300 ng/0.5 ul); Body weight for rats at time of surgery was reported, so dose could be calculated.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Single 4-minute infusion. appropriate for this study type and outcomes of interest
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	All Outcomes: the study goal was not to have a dose-dependent effect and there is only one exposure concentration with a sham control
	Metric 12:	Exposure Route and Method	Low	All Outcomes: The route of exposure was infusion of phthalic acid (in solution) into the NBM
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: The test animal species, strain, sex, and starting body weight were reported, and the test animal was obtained from a commercial source or laboratory-maintained colony. The age of rats was not reported. The test species and strain were an appropriate animal model for the evaluation of the specific outcome.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Most husbandry conditions were reported (e.g., temperature, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations. Humidity was not reported.
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The number of total animals was reported as 122 rats (18 excluded for miscannulation). The number of rats per study group is unclear.

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Study Citation:	Power, A. E., Mcgaugh, J. L. (2002). Cholinergic activation of the basolateral amygdala regulates unlearned freezing behavior in rats. Behavioural Brain Research 134(1-2):307-315.
Health Outcome(s):	Neurological/Behavioral; Nutritional/Metabolic;
Reported Health Effect(s):	Neurological/Behavioral: unconditioned fear behavior, open field activity, shock context; Nutritional/Metabolic: Body weight gain;
Duration:	Acute (less than or equal to 24 hr) single infusion
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6816161

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: The study is designed as a mechanistic study to determine if removing cholinergic input (with drugs) to basolateral amygdala (BLA) with phthalic acid -induced lesions in nucleus basalis magnocellularis (NBM) alters rats' freezing behavior in presence of cat hair; however, outcomes included comparisons between rats with phthalic acid-induced lesions to sham controls for behavioral endpoints and body weight. It may be possible that lesions induced in NBM by other mechanisms or exposures would result in similar outcomes; however, this study is specific to PA lesions. The methodology addressed the intended outcomes of interest. infusion into the NBM was the route of exposure for phthalic acid.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Details of the outcome assessment protocol were reported and outcomes appear to be assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Medium	All Outcomes: 5 animals/group were sampled for conditioned fear behavior; Open field activity: sham n=14, PA-lesion n = 10; shock context: n=14/group; weight fain: sham n=19, PA lesion n=20; it is unclear how many total animals were in each exposure group
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Not applicable for this study
	Metric 20:	Negative Control Response	High	All Outcomes: The biological responses of the sham control groups were adequate
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Although the study did not report all information to determine confounding factors, reported information did not identify differences among study groups; there was a significant decreased weight gain in treated rats compared to sham control. Water and food consumption were not measured/reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical methods were described and adequate
	Metric 24:	Reporting of Data	High	All Outcomes: Data for exposure-related findings were presented for all outcomes by exposure group. Data are reported in figures with the means and standard error bars.

Additional Comments: The study is designed as a mechanistic study to determine if removing cholinergic input (with drugs) to basolateral amygdala (BLA) with phthalic acid -induced lesions in nucleus basalis magnocellularis (NBM) alters rats' freezing behavior in presence of cat hair. infusion into the NBM was the route of exposure for phthalic acid. Outcomes included comparisons between rats with phthalic acid-induced lesions to sham controls for behavioral endpoints and body weight.

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Study Citation:	Power, A. E., Mcgaugh, J. L. (2002). Cholinergic activation of the basolateral amygdala regulates unlearned freezing behavior in rats. Behavioural Brain Research 134(1-2):307-315.
Health Outcome(s):	Neurological/Behavioral; Nutritional/Metabolic;
Reported Health Effect(s):	Neurological/Behavioral: unconditioned fear behavior, open field activity, shock context; Nutritional/Metabolic: Body weight gain;
Duration:	Acute (less than or equal to 24 hr) single infusion
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	6816161

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

Study Citation:	Product Safety Labs, (1982). Skin corrosion test with six New Zealand albino rabbits (phthalic anhydride flake).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Dermal corrosion
Duration:	Acute (less than or equal to 24 hr) 4-hour skin corrosion study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980188

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported. A sample number was provided (910-31).
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A concurrent negative control is not required for this study type.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were selected.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage of the test substance was not reported. The test substance was uniformly mixed with water and applied as a 58.6% paste. No other details were provided.
	Metric 8:	Consistency of Exposure Administration	High	The test substance appears to have been applied consistently across all animals.
	Metric 9:	Reporting of Doses/Concentrations	Medium	A nominal dose (0.5 g) was reported. The body weights of the rabbits were not reported
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration and frequency were appropriate and agreed with OECD guidelines.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	Only one dose (0.5 g) was tested. Justification for choosing this dose was not reported, however this is the suggested dose in OECD 404 guidelines.
	Metric 12:	Exposure Route and Method	Medium	A 2.5 cm2 gauze patch was used to apply 0.5 grams of test substance. This area is smaller than the recommended 6 cm2 (OECD 404). The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The animal source, species, strain were reported. Sex, initial body weight, and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Housing conditions were partially reported. Temperature and humidity were not reported although it the study does report animals were kept in an environmentally controlled room.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was appropriate (n=6).
Domain 5: Outcome Assessment				

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Study Citation:	Product Safety Labs, (1982). Skin corrosion test with six New Zealand albino rabbits (phthalic anhydride flake).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Dermal corrosion
Duration:	Acute (less than or equal to 24 hr) 4-hour skin corrosion study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980188

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Medium	The methods used to examine skin (e.g., Draize) were not reported; however, the criteria for identifying a test substance as corrosive were specified. The study only assessed animals at 4 and 48 hours. OECD TG recommend examinations at 60 minutes, and then at 24, 48 and 72 hours after patch removal, and longer if testing for reversal.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rabbits.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not required for this study type.
	Metric 20:	Negative Control Response	N/A	The negative control area was not included.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not applicable to this study type.
	Metric 24:	Reporting of Data	Medium	Corrosion results were fully reported for each animal and timepoint.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Lung/Respiratory; Hepatic/Liver; Neurological/Behavioral;
Reported Health Effect(s):	Lung/Respiratory: Gross necropsy of lungs; changes in respiration; Hepatic/Liver: Gross necropsy of liver; Neurological/Behavioral: Behavioral changes and neurological clinical signs (e.g. hypoactivity, tremors, convulsions);
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was definitively identified by name as phthalic anhydride. CASRN 85-44-9.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance and batch were reported.
	Metric 3:	Test Substance Purity	High	All Outcomes: The purity was reported to be 99.9%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A concurrent negative control was not included; however, this is an acute lethality study, and a negative control group is not required.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control was not required for the study type.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: It does not appear that the animals were randomly selected. “On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for testing.”
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	All Outcomes: Storage and preparation of the test substance were adequately reported. The test substance was stored at room temperature. Details on generating the aerosol were adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Gravimetric and nominal concentrations are reported along with particle size distribution and mass median aerodynamic diameter.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure duration (4 hours) was appropriate for the aim of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: The study conducted a limit test according to OECD 403 TG. A single concentration, representing the maximum attainable exposure level, was tested.
	Metric 12:	Exposure Route and Method	Medium	All Outcomes: Nose-only inhalation was performed. The study does not report if animals were acclimated to the apparatus prior to testing.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The animal source, species, strain, sex, age, and initial body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals/group was sufficient for the type of study (5/sex).

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Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Lung/Respiratory; Hepatic/Liver; Neurological/Behavioral;
Reported Health Effect(s):	Lung/Respiratory: Gross necropsy of lungs; changes in respiration; Hepatic/Liver: Gross necropsy of liver; Neurological/Behavioral: Behavioral changes and neurological clinical signs (e.g. hypoactivity, tremors, convulsions);
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was in consistent with OECD 403 guidelines.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Sampling was sufficient. The study reported findings for all rats (5/group).
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, outputs were either not subjective (death, body weight), clinical signs, or gross necropsy.
	Metric 20:	Negative Control Response	N/A	All Outcomes: The negative control was not required for this acute toxicity study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: The test substance is a respiratory irritant; therefore, respiratory rate should be evaluated. The study did report irregular respiration in most animals but did not indicate the severity or if the respiration was increased or decreased. Body weights were reported; however, food intake was not.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	All Outcomes: A control group was not included; therefore, statistical analysis cannot be performed.
	Metric 24:	Reporting of Data	High	Lung/Respiratory: The number of animals with respiratory clinical signs were reported; gross necropsy lung findings were reported for the male that died.; Hepatic/Liver: Gross necropsy liver findings were reported for the male that died; no gross abnormalities were observed in the other animals.; Neurological/Behavioral: Clinical signs related to neurological effects were reported for each animal.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Gross necropsy (Gross necropsy); Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Gross necropsy (Gross necropsy): Gross necropsy; Ocular/Sensory: Clinical observations of eyes (ocular discharge); Skin/Connective Tissue: Gross observation of skin and fur;
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was definitively identified by name as phthalic anhydride. CASRN 85-44-9.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance and batch were reported.
	Metric 3:	Test Substance Purity	High	All Outcomes: The purity was reported to be 99.9%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A concurrent negative control was not included; however, this is an acute lethality study, and a negative control group is not required.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control was not required for the study type.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: It does not appear that the animals were randomly selected. “On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for testing.”
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	All Outcomes: Storage and preparation of the test substance were adequately reported. The test substance was stored at room temperature. Details on generating the aerosol were adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Gravimetric and nominal concentrations are reported along with particle size distribution and mass median aerodynamic diameter.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure duration (4 hours) was appropriate for the aim of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: The study conducted a limit test according to OECD 403 TG. A single concentration, representing the maximum attainable exposure level, was tested.
	Metric 12:	Exposure Route and Method	Medium	All Outcomes: Nose-only inhalation was performed. The study does not report if animals were acclimated to the apparatus prior to testing.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The animal source, species, strain, sex, age, and initial body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals/group was sufficient for the type of study (5/sex).
Domain 5: Outcome Assessment				

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Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Gross necropsy (Gross necropsy); Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Gross necropsy (Gross necropsy): Gross necropsy; Ocular/Sensory: Clinical observations of eyes (ocular discharge); Skin/Connective Tissue: Gross observation of skin and fur;
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	Gross necropsy (Gross necropsy): The outcome methodology was sensitive to endpoint of interest.; Ocular/Sensory: The outcome methodology was in consistent with OECD 403 guidelines.; Skin/Connective Tissue: The outcome methodology was in consistent with OECD 403 guidelines.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Sampling was sufficient. The study reported findings for all rats (5/group).
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, outputs were either not subjective (death, body weight), clinical signs, or gross necropsy.
	Metric 20:	Negative Control Response	N/A	All Outcomes: The negative control was not required for this acute toxicity study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: The test substance is a respiratory irritant; therefore, respiratory rate should be evaluated. The study did report irregular respiration in most animals but did not indicate the severity or if the respiration was increased or decreased. Body weights were reported; however, food intake was not.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Gross necropsy (Gross necropsy): No gross necropsy findings were observed therefore statistical analysis is not needed.; Ocular/Sensory: A control group was not included; therefore, statistical analysis cannot be performed.; Skin/Connective Tissue: A control group was not included; therefore, statistical analysis cannot be performed.
	Metric 24:	Reporting of Data	Medium	Gross necropsy (Gross necropsy): Data are reported as negative for animals that survived the 14-day observation period. The animal that died, necropsy finding were reported for 2 organs.; Ocular/Sensory: Gross observations were reported for animals when present.; Skin/Connective Tissue: Gross observations related to fur and skin were reported for animals when present.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was definitively identified by name as phthalic anhydride. CASRN 85-44-9.
	Metric 2:	Test Substance Source	High	The source of the test substance and batch were reported.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.9%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A concurrent negative control was not included; however, this is an acute lethality study, and a negative control group is not required.
	Metric 5:	Positive Controls	N/A	A positive control was not required for the study type.
	Metric 6:	Randomized Allocation of Animals	Low	It does not appear that the animals were randomly selected. “On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for testing.”
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Storage and preparation of the test substance were adequately reported. The test substance was stored at room temperature. Details on generating the aerosol were adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	Gravimetric and nominal concentrations are reported along with particle size distribution and mass median aerodynamic diameter.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration (4 hours) was appropriate for the aim of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The study conducted a limit test according to OECD 403 TG. A single concentration, representing the maximum attainable exposure level, was tested.
	Metric 12:	Exposure Route and Method	Medium	Nose-only inhalation was performed. The study does not report if animals were acclimated to the apparatus prior to testing.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, age, and initial body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was sufficient for the type of study (5/sex).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive to the endpoint of interest. Animals were assessed at least once a day for mortality, gross evaluation and clinical signs.

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Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rats (5/group).
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, outputs were either not subjective (death, body weight) or clinical signs.
	Metric 20:	Negative Control Response	N/A	The negative control was not required for this acute toxicity study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, the respiratory rate should be evaluated. The study did report irregular respiration in most animals, but did not indicate the severity or if the respiration was increased or decreased. Body weights were reported; however, food intake was not.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss that there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was not performed; however, data is presented in such a way that independent analysis could be done.
	Metric 24:	Reporting of Data	High	Data are fully reported.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Gastrointestinal
Reported Health Effect(s):	Gastrointestinal: Fecal volume
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was definitively identified by name as phthalic anhydride. CASRN 85-44-9.
	Metric 2:	Test Substance Source	High	The source of the test substance and batch were reported.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.9%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A concurrent negative control was not included; however, this is an acute lethality study, and a negative control group is not required.
	Metric 5:	Positive Controls	N/A	A positive control was not required for the study type.
	Metric 6:	Randomized Allocation of Animals	Low	It does not appear that the animals were randomly selected. “On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for testing.”
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Storage and preparation of the test substance were adequately reported. The test substance was stored at room temperature. Details on generating the aerosol were adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	Gravimetric and nominal concentrations are reported along with particle size distribution and mass median aerodynamic diameter.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration (4 hours) was appropriate for the aim of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The study conducted a limit test according to OECD 403 TG. A single concentration, representing the maximum attainable exposure level, was tested.
	Metric 12:	Exposure Route and Method	Medium	Nose-only inhalation was performed. The study does not report if animals were acclimated to the apparatus prior to testing.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, age, and initial body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was sufficient for the type of study (5/sex).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	The frequency or method used to assess fecal volume was not reported.

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Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Gastrointestinal
Reported Health Effect(s):	Gastrointestinal: Fecal volume
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rats (5/group).
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, outputs were either not subjective (death, body weight), clinical signs, or gross necropsy.
	Metric 20:	Negative Control Response	N/A	The negative control was not required for this acute toxicity study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, respiratory rate should be evaluated. The study did report irregular respiration in most animals but did not indicate the severity or if the respiration was increased or decreased. Body weights were reported; however, food intake was not.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	A control group was not included; therefore, statistical analysis cannot be performed.
	Metric 24:	Reporting of Data	Medium	Gross observations were reported for animals when present.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was definitively identified by name as phthalic anhydride. CASRN 85-44-9.
	Metric 2:	Test Substance Source	High	The source of the test substance and batch were reported.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.9%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A concurrent negative control was not included; however, this is an acute lethality study, and a negative control group is not required.
	Metric 5:	Positive Controls	N/A	A positive control was not required for the study type.
	Metric 6:	Randomized Allocation of Animals	Low	It does not appear that the animals were randomly selected. “On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for testing.”
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Storage and preparation of the test substance were adequately reported. The test substance was stored at room temperature. Details on generating the aerosol were adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	Gravimetric and nominal concentrations are reported along with particle size distribution and mass median aerodynamic diameter.
	Metric 10:	Exposure Frequency and Duration	High	The exposure duration (4 hours) was appropriate for the aim of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The study conducted a limit test according to OECD 403 TG. A single concentration, representing the maximum attainable exposure level, was tested.
	Metric 12:	Exposure Route and Method	Medium	Nose-only inhalation was performed. The study does not report if animals were acclimated to the apparatus prior to testing.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, age, and initial body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group was sufficient for the type of study (5/sex).
Domain 5: Outcome Assessment				

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Study Citation:	PSL,, Eurofins (2010). Phthalic anhydride: Acute inhalation toxicity study in rats.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Acute (less than or equal to 24 hr) Acute 4-hour exposure
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980171

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Medium	The outcome methodology slightly deviated from guidelines. OECD 403 recommends body weights should be measured "at least on days 1, 3 and 7 (and weekly thereafter)". This study measured body weights on Day0, 7, and 14 (termination) or after death
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. The study reported findings for all rats (5/group).
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, outputs were either not subjective (death, body weight) or clinical signs.
	Metric 20:	Negative Control Response	N/A	The negative control was not required for this acute toxicity study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, respiratory rate should be evaluated. The study did report irregular respiration in most animals but did not indicate the severity or if the respiration was increased or decreased. Body weights were reported; however, food intake was not.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	A control group was not included; therefore, statistical analysis cannot be performed.
	Metric 24:	Reporting of Data	High	Data are fully reported.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Gross necropsy, lung weight, histopathology, clinical signs (blepharospasm, dyspnea)
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	The test substance purity (≥99%) was reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to aerosolized acetone for 360 min/day.
	Metric 5:	Positive Controls	Medium	Since this was a method development study, there are no known positive controls for a respiratory LLNA. The study used chemicals known to be respiratory irritants, and also conducted standard skin LLNA assays, which were used as a positive control.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	The details of aerosol preparation were described. The method and equipment used to generate the aerosol was reported. The test substance was prepared daily.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	High	Analytical and target concentrations were both reported. The variation of concentration did not deviate widely. The analytical method used to measure concentration was reported and was appropriate. The MMAD and GSD were reported.
	Metric 10:	Exposure Frequency and Duration	Medium	Test animals were exposed by inhalation daily for three days. The 3 daily exposures are consistent with the standard LLNA assay. The study used variations in exposure duration (45, 90, 180, or 360 min/day) rather than differing concentrations to investigate a dose-response relationship.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Only one concentration of the test substance was administered. Instead of multiple concentrations, different durations of exposure were used. This decision was discussed in detail and justified by the study authors. However, a linear response was not observed, indicating that increasing durations, rather than concentrations, may not be the ideal approach for identifying a dose-response.

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Gross necropsy, lung weight, histopathology, clinical signs (blepharospasm, dyspnea)
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	Animals were dosed via a nose-only chamber, which is appropriate for this test substance. The air flow rate (0.3L/min/animal) was reported. The study did not report whether animals were properly acclimated to the restraint and the exposure apparatus. This is considered to be a serious flaw because stress from restraint could trigger an immune response that may influence the LLNA results. However, the study did include sham (vehicle) controls.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male BALB/c mice. The source, sex, and age of the test animals were reported. Initial body weights were not reported, but this is unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animals had free access to food and water and were kept “under conventional laboratory conditions.” No other husbandry details were provided.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 6 animals per group. The vehicle control group consisted of 12 animals.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Respiratory outcomes assessment methodologies were sensitive and appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the respiratory outcome assessment protocols, including gross necropsy, lung weights, histopathology, and clinical signs, were reported and assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Medium	All of the animals used in the study were observed for signs of toxicity daily. While most respiratory outcomes were assessed in all test animals, histopathology was performed in the high dose group only.
	Metric 19:	Blinding of Assessors	N/A	The study did not report whether assessors were blinded to treatment group for the gross necropsy or assessment of clinical signs, and this is not likely to have a substantial impact on results. Blinding is not required for the assessment of cell proliferation, lung weight, or histopathology.
	Metric 20:	Negative Control Response	Low	Histopathology results of controls were not reported in the data table or described in the text. Quantitative data were also not provided for lung weights or gross necropsy results. Insufficient information is provided to assess whether the control responses are appropriate.
Domain 6: Confounding / Variable Control				

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Gross necropsy, lung weight, histopathology, clinical signs (blepharospasm, dyspnea)
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Respiratory rates were not measured, and the test substance is a known respiratory irritant. The study authors indicated that the "total dose that reached the airway epithelium may be much lower due to the altered breathing frequency and pattern induced by allergen-induced irritation to the respiratory epithelium." The presence of air in the gastrointestinal tract was "suggestive of extensive irritation." The authors also noted that the calculated respiratory ED3 values assumed a normal breathing rate and, therefore, may be inaccurate.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Uninformative	Statistical analysis of lung weight was not performed; an effect was reported, and data enabling an independent statistical analysis were not provided. Statistical analysis of clinical signs and gross necropsy was likely not necessary (negative results were reported qualitatively in the text). Statistical analysis of histopathology data cannot be performed because the control responses are not reported.
	Metric 24:	Reporting of Data	Low	Exposure-related findings for lung weights and negative findings for gross necropsy and clinical signs were qualitatively described in the text. Histopathology results were reported for the treatment groups only, and severity scores were described.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	The test substance purity (≥99%) was reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to acetone (vehicle) for 360 min/day.
	Metric 5:	Positive Controls	Medium	Since this was a methods development study, there are no known positive controls for a respiratory LLNA. The study used chemicals known to be respiratory irritants, and also conducted standard skin LLNA assays, which were used as a positive control.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	The details of aerosol preparation were described. The method and equipment used to generate the aerosol was reported. The test substance was prepared daily.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	High	Analytical and target concentrations were both reported. The variation of concentration did not deviate widely. The analytical method used to measure concentration was reported and was appropriate. The MMAD and GSD were reported.
	Metric 10:	Exposure Frequency and Duration	Medium	Test animals were exposed by inhalation daily for three days. The 3 daily exposures is consistent with the standard LLNA assay. The study used variations in exposure duration (45, 90, 180, or 360 min/day) rather than differing concentrations to investigate a dose-response relationship.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only one concentration of the test substance was administered. Instead of multiple concentrations, different durations of exposure were used. This decision was discussed in detail and justified by the study authors. However, a linear response was not observed, indicating that increasing durations, rather than concentrations, may not be the ideal approach for identifying a dose-response.
	Metric 12:	Exposure Route and Method	Low	Animals were dosed via a nose-only chamber, which is appropriate for this test substance. The air flow rate (0.3L/min/animal) was reported. The study did not report whether animals were properly acclimated to restraint and the exposure apparatus. This is considered to be a serious flaw because stress from restraint could trigger an immune response that may influence the LLNA results. However, the study did include sham (vehicle) controls.

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male BALB/c mice. The source, sex, and age of the test animals were reported. Initial body weights were not reported, but this is unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animals had free access to food and water and were kept “under conventional laboratory conditions.” No other husbandry details were provided.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 6 animals per group. The vehicle control group consisted of 12 animals.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology and frequency of measurements were reported and sensitive to the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Mortality was assessed in all test animals according to the same protocol and time intervals.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were weighed before dosing and at the conclusion of the study period (3 days after the last exposure).
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Respiratory rates were not measured, and the test substance is a known respiratory irritant. The study authors indicated that the “total dose that reached the airway epithelium may be much lower due to the altered breathing frequency and pattern induced by allergen-induced irritation to the respiratory epithelium.” The presence of air in the gastrointestinal tract was “suggestive of extensive irritation.” The authors also noted that the calculated respiratory ED3 values assumed a normal breathing rate and, therefore, may be inaccurate.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not necessary, as there was no mortality across all test groups.
	Metric 24:	Reporting of Data	High	Mortality was reported qualitatively, which was acceptable because there were no treatment-related mortalities.

Additional Comments: None

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

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

Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Lymph node cell proliferation
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	The test substance purity (≥99%) was reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to acetone (vehicle)for 360 min/day.
	Metric 5:	Positive Controls	Medium	Since this was a methods development study, there are no known positive controls for a respiratory LLNA. The study used chemicals known to be respiratory irritants, and also conducted standard skin LLNA assays, which were used as a positive control.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	The details of aerosol preparation were described. The method and equipment used to generate the aerosol was reported. The test substance was prepared daily.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	High	Analytical and target concentrations were both reported. The variation of concentration did not deviate widely. The analytical method used to measure concentration was reported and was appropriate. The MMAD and GSD were reported.
	Metric 10:	Exposure Frequency and Duration	Medium	Test animals were exposed by inhalation daily for three days. The 3 daily exposures are consistent with the standard LLNA assay. The study used variations in exposure duration (45, 90, 180, or 360 min/day) rather than differing concentrations to investigate a dose-response relationship.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only one concentration of the test substance was administered. Instead of multiple concentrations, different durations of exposure were used. This decision was discussed in detail and justified by the study authors. However, a linear response was not observed, indicating that increasing durations, rather than concentrations, may not be the ideal approach for identifying a dose-response.
	Metric 12:	Exposure Route and Method	Low	The air flow rate (0.3L/min/animal) was reported. The study did not report whether animals were properly acclimated to restraint and the exposure apparatus. This is considered to be a serious flaw because stress from restraint could trigger an immune response that may influence the LLNA results. However, the study did include sham (vehicle) controls.

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Lymph node cell proliferation
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male BALB/c mice. The source, sex, and age of the test animals were reported. Initial body weights were not reported, but this is unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animals had free access to food and water and were kept “under conventional laboratory conditions.” No other husbandry details were provided.
	Metric 15:	Number of Animals per Group	Medium	The number of animals was adequate. The study used 6 animals per group. The vehicle control group consisted of 12 animals.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	A modified LLNA using ex vivo labeling of the proliferating LN cells was used, which deviates from the OECD LLNA guidelines. However, the methodology was justified by the study authors and was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the lymph node cell proliferation assessment protocol were reported and assessed consistently across groups.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were evaluated for lymph node cell proliferation.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Respiratory rates were not measured, and the test substance is a known respiratory irritant. The study authors indicated that the “total dose that reached the airway epithelium may be much lower due to the altered breathing frequency and pattern induced by allergen-induced irritation to the respiratory epithelium.” The presence of air in the gastrointestinal tract was “suggestive of extensive irritation.” The authors also noted that the calculated respiratory ED3 values assumed a normal breathing rate and, therefore, may be inaccurate.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	Stimulation indices were presented for each exposure group.

Additional Comments: None

Overall Quality Determination High

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Lymph node cell proliferation
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric	Rating	Comments

Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Nutritional/Metabolic; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness));
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Clinical signs (piloerection, hunched posture, sluggishness);
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	All Outcomes: The test substance purity (≥99%) was reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: The study included a concurrent negative control group that was exposed to acetone (vehicle) for 360 min/day.
	Metric 5:	Positive Controls	Medium	All Outcomes: Since this was a methods development study, there are no known positive controls for a respiratory LLNA. The study used chemicals known to be respiratory irritants, and also conducted standard skin LLNA assays, which were used as a positive control.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	All Outcomes: The details of aerosol preparation were described. The method and equipment used to generate the aerosol was reported. The test substance was prepared daily.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Details of exposure administration were reported and exposures were administered consistently across study animals.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Analytical and target concentrations were both reported. The variation of concentration did not deviate widely. The analytical method used to measure concentration was reported and was appropriate. The MMAD and GSD were reported.
	Metric 10:	Exposure Frequency and Duration	Medium	Nutritional/Metabolic: Test animals were exposed by inhalation daily for three days. The 3 daily exposures is consistent with the standard LLNA assay. The study used variations in exposure duration (45, 90, 180, or 360 min/day) rather than differing concentrations to investigate a dose-response relationship.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Test animals were exposed by inhalation daily for three days. The 3 daily exposures are consistent with the standard LLNA assay. The study used variations in exposure duration (45, 90, 180, or 360 min/day) rather than differing concentrations to investigate a dose-response relationship.

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Nutritional/Metabolic; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness));
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Clinical signs (piloerection, hunched posture, sluggishness);
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: Only one concentration of the test substance was administered. Instead of multiple concentrations, different durations of exposure were used. This decision was discussed in detail and justified by the study authors. However, a linear response was not observed, indicating that increasing durations, rather than concentrations, may not be the ideal approach for identifying a dose-response.
	Metric 12:	Exposure Route and Method	Low	All Outcomes: Animals were dosed via a nose-only chamber, which is appropriate for this test substance. The air flow rate (0.3L/min/animal) was reported. The study did not report whether animals were properly acclimated to restraint and the exposure apparatus. This is considered to be a serious flaw because stress from restraint could trigger an immune response that may influence the LLNA results. However, the study did include sham (vehicle) controls.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: The study used male BALB/c mice. The source, sex, and age of the test animals were reported. Initial body weights were not reported, but this is unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	All Outcomes: Animals had free access to food and water and were kept “under conventional laboratory conditions.” No other husbandry details were provided.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals was adequate. The study used 6 animals per group. The vehicle control group consisted of 12 animals.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Nutritional/Metabolic: The outcome methodology and frequency of measurements were reported and sensitive to the outcome of interest.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Test animals were observed daily during exposure and for 3 days following the last exposure for mortality and signs of toxicity.
	Metric 17:	Consistency of Outcome Assessment	High	Nutritional/Metabolic: Test animal body weights were examined consistently across groups.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Signs of toxicity were assessed in all test animals according to the same protocol and time intervals.
	Metric 18:	Sampling Adequacy	High	Nutritional/Metabolic: All of the animals used in the study were weighed before dosing and at the conclusion of the study period (3 days after the last exposure).; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): All of the animals used in the study were observed for signs of toxicity daily.
	Metric 19:	Blinding of Assessors	N/A	Nutritional/Metabolic: Blinding is not required for this endpoint.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): The study did not report whether assessors were blinded to treatment group for evaluating clinical signs, and this is not likely to have a substantial impact on results.

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Nutritional/Metabolic; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness));
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Clinical signs (piloerection, hunched posture, sluggishness);
Duration:	Short-term (>1-30 days) Short-term inhalation LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
	Metric 20:	Negative Control Response	Low	Nutritional/Metabolic: Negative control responses were not provided.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): The biological response of the negative control group was not reported.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Nutritional/Metabolic: Respiratory rates were not measured, and the test substance is a known respiratory irritant. The study authors indicated that the "total dose that reached the airway epithelium may be much lower due to the altered breathing frequency and pattern induced by allergen-induced irritation to the respiratory epithelium." The presence of air in the gastrointestinal tract was "suggestive of extensive irritation." The authors also noted that the calculated respiratory ED3 values assumed a normal breathing rate and, therefore, may be inaccurate.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Respiratory rates were not measured and the test substance is a known respiratory irritant. The study authors indicated that the "total dose that reached the airway epithelium may be much lower due to the altered breathing frequency and pattern induced by allergen-induced irritation to the respiratory epithelium." The presence of air in the gastrointestinal tract was "suggestive of extensive irritation." The authors also noted that the calculated respiratory ED3 values assumed a normal breathing rate and, therefore, may be inaccurate.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Uninformative	Nutritional/Metabolic: Statistical analysis of body weights was not performed; effects were reported, and data enabling an independent statistical analysis were not provided.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Statistical analysis of clinical signs was not reported. Effects were observed and data were not provided for an independent analysis.
	Metric 24:	Reporting of Data	Low	Nutritional/Metabolic: Body weight data were not shown for each exposure group, but treatment-related results were described in the text.; Clinical signs (Clinical signs (piloerection, hunched posture, sluggishness)): Clinical signs were not described for each exposure group, but treatment-related effects were described in the text. Control responses were not reported.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Lymph node cell proliferation
Duration:	Short-term (>1-30 days) Short-term dermal LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	The test substance purity (≥99%) was reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to a vehicle control.
	Metric 5:	Positive Controls	Medium	Positive controls included test substances previously shown to demonstrate positive results in standard LLNA assays.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The test substance was dissolved in a 4:1 (vol/vol) mixture of acetone and olive oil. Mixing, frequency of preparation, homogeneity, and stability were not reported. However, given that the study was short-term, this is unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure administration details were reported. 25 μL of the vehicle or test solutions was applied to the dorsum of both ears for three consecutive days. It is unclear if measures were taken to minimize the potential for oral exposure.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal amount of test substance applied was reported. Animals were administered 25 µl of test solution containing an unspecified amount of the test substance in a 4:1 (vol/vol) mixture of acetone and olive oil. The concentration was reported to be 25%. Body weight was not reported, so a mg/kg dose cannot be estimated.
	Metric 10:	Exposure Frequency and Duration	Medium	Test animals were exposed via dermal application to the dorsum of both ears for three consecutive days. This is consistent with the current OECD 429 TG.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	The study tested a single exposure group which was justified by the study authors. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	Animals were dermally exposed on the dorsal side of both ears for 3 consecutive days, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The study used male BALB/c mice. The source, sex, and age of the test animals were reported. Initial body weights were not reported, but this is unlikely to have a substantial impact on results.

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Study Citation:	Arts, J. H., Jong, de, W. H., Triel, van, J. J., Schijf, M. A., Klerk, de, A., Loveren, van, H., Kuper, C. F. (2008). The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106(2):423-434.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Lymph node cell proliferation
Duration:	Short-term (>1-30 days) Short-term dermal LLNA
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1222879; Linked HERO ID(s): 1222879, 652441

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animals had free access to food and water and were kept “under conventional laboratory conditions.” No other husbandry details were provided.
	Metric 15:	Number of Animals per Group	Low	The study used 3 animals per group. OECD guidelines recommend a minimum of 4 animals per group for the LLNA assay. However, 3 animals per group is sufficient for statistical analysis.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	A modified LLNA using ex vivo labeling of the proliferating LN cells was used, which deviates from the OECD LLNA guidelines. However, the methodology was sensitive and appropriate for the outcome of interest. Other outcomes (not related to sensitization) that are typically assessed in an LLNA assay were not performed.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the LLNA assessment protocol were reported and assessed consistently across groups.
	Metric 18:	Sampling Adequacy	High	All of the animals used in the study were evaluated for lymph node cell proliferation.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	Low	The proliferative responses of the negative control group were not reported.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	The method to calculate an effective concentration 3% (EC3) was reported and appropriate.
	Metric 24:	Reporting of Data	Medium	Proliferation results were not reported. Stimulation indicies were provided.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Ban, M., Hettich, D. (2005). Effect of Th2 cytokine antagonist treatments on chemical-induced allergic response in mice. Journal of Applied Toxicology 25(3):239-247.
Health Outcome(s):	Immune/Hematological; Sensitization;
Reported Health Effect(s):	Immune/Hematological: Serum IgG2a and total IgE .IL-2, IL-4, IL-10 and IFN-g in supernatants of spleen cell culture with concanavalin A.; Sensitization: Th2 response, leading to increased risk of asthma like syndrome in mice.;
Duration:	Short-term (>1-30 days) Short term 11 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	83939

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Test substance was identified as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of test substance was identified as Aldrich Chemical Co, France. Batch/lot number was not provided.
	Metric 3:	Test Substance Purity	High	All Outcomes: The purity of the test substance was reported as 97%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A negative control group was included and appropriate.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: The animals were divided randomly into groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Preparation and storage were not described. Given the chemical properties of the test substance, this is unlikely to have a substantial effect on the results.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Exposure was consistent across the study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	All Outcomes: Body weights were not reported.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: Only one dose studied, effect was seen at this dose.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The exposure route was appropriate (dermal).
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: Starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Husbandry conditions were reported and adequate.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals treated per group was appropriate.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: Methodology was appropriate for outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Low	All Outcomes: Details regarding sampling of outcomes were not reported.

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Study Citation:	Ban, M., Hettich, D. (2005). Effect of Th2 cytokine antagonist treatments on chemical-induced allergic response in mice. Journal of Applied Toxicology 25(3):239-247.
Health Outcome(s):	Immune/Hematological; Sensitization;
Reported Health Effect(s):	Immune/Hematological: Serum IgG2a and total IgE .IL-2, IL-4, IL-10 and IFN-g in supernatants of spleen cell culture with concanavalin A.; Sensitization: Th2 response, leading to increased risk of asthma like syndrome in mice.;
Duration:	Short-term (>1-30 days) Short term 11 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	83939

Domain	Metric		Rating	Comments
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	High	All Outcomes: The negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: No information was provided to either to support or dismiss differences in groups in health outcomes or attrition
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	All Outcomes: Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Fabro, S., Shull, G., Brown, N. A. (1982). The relative teratogenic index and teratogenic potency: proposed components of developmental toxicity risk assessment. Teratogenesis, Carcinogenesis, and Mutagenesis 2(1):61-76.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Adult lethality
Duration:	Short-term (>1-30 days) 3 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63760

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance is identified as phthalic anhydride
	Metric 2:	Test Substance Source	High	Test substance was obtained from Eastman Kodak Company (Rochester, NY)
	Metric 3:	Test Substance Purity	High	Test substances was determined to have purity >98%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	Controls were not used or required for lethality testing
	Metric 5:	Positive Controls	N/A	Not necessary for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	PAD was prepared as a suspension in 0.5% (w/v) carboxymethyl cellulose solution immediately prior to injection to minimize hydrolysis. PAD hydrolyses to phthalic acid with a half-life on the order of seconds to minutes. Given the rapid rate of hydrolysis, there is concern that the chosen vehicle was not appropriate for PAD.
	Metric 8:	Consistency of Exposure Administration	High	Adult female mice were treated with 3 consecutive daily ip injections (0.01 ml/gm BW) of phthalic anhydride
	Metric 9:	Reporting of Doses/Concentrations	Uninformative	Study authors state that at least 5 doses were used per compound (study reports 8 compounds tested). However, the tested doses of phthalic anhydride were not reported.
	Metric 10:	Exposure Frequency and Duration	High	Lethality was evaluated after 3 consecutive daily ip injections. Animals were monitored for up to 14 days.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Uninformative	Study authors state that at least 5 doses were used per compound (study reports 8 compounds tested). However, the exact number of dose groups for phthalic anhydride were not reported (nor were the precise doses administered). Without this information the adequacy of dose spacing cannot be evaluated.
	Metric 12:	Exposure Route and Method	Medium	The test substance was administered via ip injection. The relevance of this route of exposure to humans is questionable.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Female CD-1 mice (Charles River Laboratories, Wilmington, MA) were used throughout the study. Age and starting body weight were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Study authors state that mice were housed in climate-controlled conditions (20°C, 50% humidity, 0600-1800 hr light cycle) with free access to water and NIH 31 chow.

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Study Citation:	Fabro, S., Shull, G., Brown, N. A. (1982). The relative teratogenic index and teratogenic potency: proposed components of developmental toxicity risk assessment. Teratogenesis, Carcinogenesis, and Mutagenesis 2(1):61-76.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Adult lethality
Duration:	Short-term (>1-30 days) 3 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63760

Domain	Metric		Rating	Comments
	Metric 15:	Number of Animals per Group	Medium	Authors state that at least 10 female mice were included per dose group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	For lethality testing, authors state that deaths were recorded for up to 14 days after the final ip injection. Clinical signs of toxicity were not reported, nor is it clear if study authors made these observations.
	Metric 17:	Consistency of Outcome Assessment	Medium	Animals were monitored for mortality for up to 14 days after the final injection. Study authors do not state how frequently (i.e., daily, twice daily, or every other day) animals were observed, however, this is unlikely to substantially impact results.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling.
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the outcome being assessed.
	Metric 20:	Negative Control Response	N/A	Negative controls were not included in the lethality study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Body weight changes, food/water intake and differences in use of surgery were not reported, however, lack of this information is not anticipated to substantially impact lethality testing results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods were clearly described and were appropriate for the dataset(s).
	Metric 24:	Reporting of Data	Low	Adult lethality data was presented as LD1 and LD50 values with 95% CI calculated. Data on lethality was not presented for each individual dose tested, nor were the tested doses reported.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Jha, A. M., Singh, A. C., Bharti, M. (1998). Germ cell mutagenicity of phthalic acid in mice. Mutation Research 422(2):207-212.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: -Sperm head abnormality-Dominant lethal mutation
Duration:	Short-term (>1-30 days) Acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1336719

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	The source of test substance was not reported. Lot/Batch number was not reported.
	Metric 3:	Test Substance Purity	High	The purity of the test substance was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A negative control group was included and appropriate.
	Metric 5:	Positive Controls	N/A	Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Preparation and storage were partially described. Given the chemical properties of the test substance, this is unlikely to have a substantial effect on the results.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was consistent across the study groups
	Metric 9:	Reporting of Doses/Concentrations	High	Exposure was consistent across the study groups
	Metric 10:	Exposure Frequency and Duration	High	Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	A NOAEL was not determined.
	Metric 12:	Exposure Route and Method	High	The exposure route was appropriate.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Not all husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals treated per group was appropriate
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Methodology was appropriate for outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate
	Metric 19:	Blinding of Assessors	Medium	Slides were coded.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.

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Study Citation:	Jha, A. M., Singh, A. C., Bharti, M. (1998). Germ cell mutagenicity of phthalic acid in mice. Mutation Research 422(2):207-212.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: -Sperm head abnormality-Dominant lethal mutation
Duration:	Short-term (>1-30 days) Acute
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1336719

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance identified as PA; CASRN and MW were provided.
	Metric 2:	Test Substance Source	Low	The test substance was purchased from a commercial source, but was not analytically verified.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Corn oil (vehicle control)
	Metric 5:	Positive Controls	N/A	Not needed for study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were randomly allocated to groups based on their body weights
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Preparation and storage were not described.
	Metric 8:	Consistency of Exposure Administration	Low	Gavage volume not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	Dosing reported without ambiguity
	Metric 10:	Exposure Frequency and Duration	High	14-day repeat-dose toxicity studies are often used for range-finding studies, but in this case, it was used to compare the short-term toxicity of several PEs. It was appropriate for the goals of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Single dose precludes the ability to evaluate a dose-response. Purpose of paper was to compare short-term toxicity of multiple phthalate esters, so a single dose was appropriate for the purposes of the study.
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were suited to the test substance (see above)
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, age, sex and source were reported. Initial body weights were measured.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number of Animals per Group	Medium	5-6 animals/group was sufficient for statistical analysis

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Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Mortality
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Not necessary for outcomes of interest
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	not necessary (clearly negative findings across all groups
	Metric 24:	Reporting of Data	High	A negative finding for all groups was reported in the text

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Lipids; Lung/Respiratory; Thyroid; Hepatic/Liver; Reproductive/Developmental; Endocrine (Endocrine (Adrenals)); Renal/Kidney; Cardiovascular; Immune/Hematological; Nutritional/Metabolic (Body Weight);
Reported Health Effect(s):	Lipids: Total cholesterol, TG, HDLc; Lung/Respiratory: Organ weight; Thyroid: Organ weight; Hepatic/Liver: Organ weight; clinical chemistry (AST, ALT, ALP, GGT, ALB, TP, Bilirubin, LDH, glucose); Reproductive/Developmental: Organ weight (testis and epidiymis); Endocrine (Endocrine (Adrenals)): Adrenals (organ weight); Renal/Kidney: Organ weight, serum chemistry (BUN, creatinine, electrolytes [NA, K, CL], Ca), urinalysis; Cardiovascular: Organ weight, CPK; Immune/Hematological: Organ weight (spleen, thymus); hematology; Nutritional/Metabolic (Body Weight): Body weights and food consumption;
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Test substance identified as PA; CASRN and MW were provided.
	Metric 2:	Test Substance Source	Low	All Outcomes: The test substance was purchased from a commercial source, but was not analytically verified.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity and/or grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Corn oil (vehicle control)
	Metric 5:	Positive Controls	N/A	All Outcomes: Not needed for study type
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Animals were randomly allocated to groups based on their body weights
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: Preparation and storage were not described.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: Gavage volume not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Dosing reported without ambiguity
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: 14-day repeat-dose toxicity studies are often used for range-finding studies, but in this case, it was used to compare the short-term toxicity of several PEs. It was appropriate for the goals of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: Single dose precludes the ability to evaluate a dose-response. Purpose of paper was to compare short-term toxicity of multiple phthalate esters, so a single dose was appropriate for the purposes of the study.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The route and method of exposure were reported and were suited to the test substance (see above)
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: Species, strain, age, sex and source were reported. Initial body weights were measured.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure.

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Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Lipids; Lung/Respiratory; Thyroid; Hepatic/Liver; Reproductive/Developmental; Endocrine (Endocrine (Adrenals)); Renal/Kidney; Cardiovascular; Immune/Hematological; Nutritional/Metabolic (Body Weight);
Reported Health Effect(s):	Lipids: Total cholesterol, TG, HDLc; Lung/Respiratory: Organ weight; Thyroid: Organ weight; Hepatic/Liver: Organ weight; clinical chemistry (AST, ALT, ALP, GGT, ALB, TP, Bilirubin, LDH, glucose); Reproductive/Developmental: Organ weight (testis and epidiymis); Endocrine (Endocrine (Adrenals)): Adrenals (organ weight); Renal/Kidney: Organ weight, serum chemistry (BUN, creatinine, electrolytes [NA, K, CL], Ca), urinalysis; Cardiovascular: Organ weight, CPK; Immune/Hematological: Organ weight (spleen, thymus); hematology; Nutritional/Metabolic (Body Weight): Body weights and food consumption;
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric		Rating	Comments
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: 5-6 animals/group was sufficient for statistical analysis
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups
	Metric 18:	Sampling Adequacy	High	All Outcomes: Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Not necessary for outcomes of interest
	Metric 20:	Negative Control Response	High	All Outcomes: The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical methods (including any calculations or data transformations) were clearly described

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Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Lipids; Lung/Respiratory; Thyroid; Hepatic/Liver; Reproductive/Developmental; Endocrine (Endocrine (Adrenals)); Renal/Kidney; Cardiovascular; Immune/Hematological; Nutritional/Metabolic (Body Weight);
Reported Health Effect(s):	Lipids: Total cholesterol, TG, HDLc; Lung/Respiratory: Organ weight; Thyroid: Organ weight; Hepatic/Liver: Organ weight; clinical chemistry (AST, ALT, ALP, GGT, ALB, TP, Bilirubin, LDH, glucose); Reproductive/Developmental: Organ weight (testis and epidiymis); Endocrine (Endocrine (Adrenals)): Adrenals (organ weight); Renal/Kidney: Organ weight, serum chemistry (BUN, creatinine, electrolytes [NA, K, CL], Ca), urinalysis; Cardiovascular: Organ weight, CPK; Immune/Hematological: Organ weight (spleen, thymus); hematology; Nutritional/Metabolic (Body Weight): Body weights and food consumption;
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric	Rating	Comments
	Metric 24: Reporting of Data	High	Lipids: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Lung/Respiratory: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Thyroid: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Hepatic/Liver: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Reproductive/Developmental: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Endocrine (Endocrine (Adrenals)): Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Renal/Kidney: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Cardiovascular: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Immune/Hematological: Data were clearly reported including means and measures of variance. Statistical significance is clearly reported.; Nutritional/Metabolic (Body Weight): Data were reported graphically, but included means and measures of variance. Statistical significance was noted.

Additional Comments: No effects observed

Overall Quality Determination High

Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance identified as PA; CASRN and MW were provided.
	Metric 2:	Test Substance Source	Low	The test substance was purchased from a commercial source, but was not analytically verified.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Corn oil (vehicle control)
	Metric 5:	Positive Controls	N/A	Not needed for study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were randomly allocated to groups based on their body weights
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Preparation and storage were not described.
	Metric 8:	Consistency of Exposure Administration	Low	Gavage volume not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	Dosing reported without ambiguity
	Metric 10:	Exposure Frequency and Duration	High	14-day repeat-dose toxicity studies are often used for range-finding studies, but in this case, it was used to compare the short-term toxicity of several PEs. It was appropriate for the goals of the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Single dose precludes the ability to evaluate a dose-response. Purpose of paper was to compare short-term toxicity of multiple phthalate esters, so a single dose was appropriate for the purposes of the study.
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were suited to the test substance (see above)
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, age, sex and source were reported. Initial body weights were measured.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number of Animals per Group	Medium	5-6 animals/group was sufficient for statistical analysis
Domain 5: Outcome Assessment				

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Study Citation:	Kwack, S. J., Han, E. Y., Park, J. S., Bae, J. Y., Ahn, I. Y., Lim, S. K., Kim, D. H., Jang, D. E., Choi, L., Lim, H. J., Kim, T. H., Patra, N., Park, K. L., Kim, H. S., Lee, B. M. (2010). Comparison of the short term toxicity of phthalate diesters and monoesters in Sprague-Dawley male rats. Toxicological Research 26(1):75-82.
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs
Duration:	Short-term (>1-30 days) 14-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	792143

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Not necessary for outcomes of interest
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Uninformative	Statistical analysis was not performed and data enabling independent analysis were not provided.
	Metric 24:	Reporting of Data	Uninformative	Data for clinical signs were not adequately reported; results for specific PEs are not clearly defined. Incidence data are not provided.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: mortality (no significant effect)
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Phthalic acid (88-93-3)
	Metric 2:	Test Substance Source	Low	Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported; not analytically verified.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Vehicle control was tested (corn oil)
	Metric 5:	Positive Controls	N/A	this study type does not require a positive control
	Metric 6:	Randomized Allocation of Animals	Medium	"animals were randomly allocated to groups based on their body weight"
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Information on preparation and storage was not reported
	Metric 8:	Consistency of Exposure Administration	Low	Details of exposure administration are incompletely reported; time of day and gavage volumes were not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	Oral gavage administered doses were reported without ambiguity; 250 mg/kg/day
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest; 28-day study
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. Study authors do not justify choice of single tested dose level.
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were suited to the test substance; oral gavage.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The test animal species, strain, sex, age were reported. Initial body weights were reported; however, body weight was reported in the results beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.

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Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: mortality (no significant effect)
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations.
	Metric 15:	Number of Animals per Group	Low	The number of animals per study group is unclear, but at least 6 animals per group were included (based on the number of animals evaluated).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Examined for mortality daily throughout the study
	Metric 17:	Consistency of Outcome Assessment	High	the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Medium	6/males/group were sampled; it is unclear how many total animals were in each exposure group
	Metric 19:	Blinding of Assessors	N/A	Not applicable for this study
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding factors, reported information did not identify differences among study groups; there was no significant differences in food consumption between treatment group and control. Water consumption was not measured/reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure
	Metric 23:	Data Presentation and Analysis	N/A	No statistics was needed
	Metric 24:	Reporting of Data	Low	Data for exposure-related mortality was reported in the text. It is stated that “No animals died during the PE administration period until 4 wk (data not shown)”, however data is not shown.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Nutritional/Metabolic; Cardiovascular; Reproductive/Developmental;
Reported Health Effect(s):	Nutritional/Metabolic: body weight (reduced BW), food consumption (no significant effect); Cardiovascular: Relative heart weight (no effect); Reproductive/Developmental: Relative testes weight (no effect), Relative epididymis weight (no effect), sperm count (no effect) and motility (reduced curvilinear velocity);
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Phthalic acid (88-93-3)
	Metric 2:	Test Substance Source	Low	Nutritional/Metabolic: Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported; not analytically verified; Cardiovascular: Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported; not analytically verified.; Reproductive/Developmental: Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported; not analytically verified.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Vehicle control was tested (corn oil)
	Metric 5:	Positive Controls	N/A	All Outcomes: this study type does not require a positive control
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: "animals were randomly allocated to groups based on their body weight"
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: Information on preparation and storage was not reported
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: Details of exposure administration are incompletely reported; time of day and gavage volumes were not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Oral gavage administered doses were reported without ambiguity; 250 mg/kg/day
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest; 28-day study

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Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Nutritional/Metabolic; Cardiovascular; Reproductive/Developmental;
Reported Health Effect(s):	Nutritional/Metabolic: body weight (reduced BW), food consumption (no significant effect); Cardiovascular: Relative heart weight (no effect); Reproductive/Developmental: Relative testes weight (no effect), Relative epididymis weight (no effect), sperm count (no effect) and motility (reduced curvilinear velocity);
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Nutritional/Metabolic: Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. The selected dose level was not justified; Cardiovascular: Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. Justification for selection of the single dose level was not provided.; Reproductive/Developmental: Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. Justification for selection of tested dose group was not provided.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The route and method of exposure were reported and were suited to the test substance; oral gavage.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Nutritional/Metabolic: The test animal species, strain, sex, age were reported. Initial body weights were reported; however, body weight was reported in the results beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.; Cardiovascular: The test animal species, strain, sex, age were reported. initial body weight was reported in the results beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.; Reproductive/Developmental: The test animal species, strain, sex, age were reported. Initial body weight was reported in the results beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations.
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The number of animals per study group is unclear, but at least 6 animals per group were included (based on the number of animals evaluated).
Domain 5: Outcome Assessment				

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Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Nutritional/Metabolic; Cardiovascular; Reproductive/Developmental;
Reported Health Effect(s):	Nutritional/Metabolic: body weight (reduced BW), food consumption (no significant effect); Cardiovascular: Relative heart weight (no effect); Reproductive/Developmental: Relative testes weight (no effect), Relative epididymis weight (no effect), sperm count (no effect) and motility (reduced curvilinear velocity);
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	Nutritional/Metabolic: Bd. Wt weighed on days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 28; food consumption was measured at the beginning of treatment and 2x/wk for duration of treatment period; Cardiovascular: Organ weights measured at day 28; Reproductive/Developmental: Organ weights measured at day 28; The outcome assessment methodology addressed the intended outcomes of interest and the methodology was appropriate for the outcome.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Medium	All Outcomes: 6/males/group were sampled; it is unclear how many total animals were in each exposure group
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Not applicable for this study
	Metric 20:	Negative Control Response	High	All Outcomes: The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Although the study did not report all information to determine confounding factors, reported information did not identify differences among study groups; there was no significant differences in food consumption between treatment group and control. Water consumption was not measured/reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical methods were described and adequate for comparison
	Metric 24:	Reporting of Data	High	Nutritional/Metabolic: body weight was reported (in figure) for all time-points evaluated; food consumption was noted to have not been significantly different from controls in the text (no data shown); Cardiovascular: Data for exposure-related findings were presented for outcomes by exposure group with mean and standard deviation.; Reproductive/Developmental: Data for exposure-related findings were presented for outcomes by exposure group with mean and standard deviation.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Hepatic/Liver; Renal/Kidney; Immune/Hematological;
Reported Health Effect(s):	Hepatic/Liver: Relative liver weight (no significant effect), biochemical serum analysis (no effect on any parameter); Renal/Kidney: Relative kidney weight (no effect), relative adrenal weight (no effect), serum chemistry (BUN, creatinine, NA, K, CL, Ca), urinalysis; Immune/Hematological: Relative spleen weight (no effect), relative thymus weight (no effect), hematology (no hematological parameters effected);
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Phthalic acid (88-93-3)
	Metric 2:	Test Substance Source	Low	Hepatic/Liver: Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported.; Renal/Kidney: Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported; not analytically verified.; Immune/Hematological: Chemicals used in the study were noted to have been Purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI),Fluka (Steinheim, Germany), Wako (Osaka, Japan) or TokyoKasei Kogyo Co. (TKK, Tokyo)"; it is not specified which source phthalic acid was from; no batch number was reported; not analytically verified.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Vehicle control was tested (corn oil)
	Metric 5:	Positive Controls	N/A	All Outcomes: this study type does not require a positive control
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: "animals were randomly allocated to groups based on their body weight"
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: Information on preparation and storage was not reported
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: Details of exposure administration are incompletely reported; time of day and gavage volumes were not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Oral gavage administered doses were reported without ambiguity; 250 mg/kg/day
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest; 28-day study

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Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Hepatic/Liver; Renal/Kidney; Immune/Hematological;
Reported Health Effect(s):	Hepatic/Liver: Relative liver weight (no significant effect), biochemical serum analysis (no effect on any parameter); Renal/Kidney: Relative kidney weight (no effect), relative adrenal weight (no effect), serum chemistry (BUN, creatinine, NA, K, CL, Ca), urinalysis; Immune/Hematological: Relative spleen weight (no effect), relative thymus weight (no effect), hematology (no hematological parameters effected);
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Hepatic/Liver: Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. Justification for selection of the single dose level was not provided.; Renal/Kidney: Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. Justification for selection of tested dose group was not provided.; Immune/Hematological: Only 1 dose (250 mg/kg/day) and the control was tested; single dose precludes ability to evaluate a dose response; test was designed to be compare effects among different phthalate esters and metabolites, so single dose appropriate for purposes of the study. Justification for selection of tested dose group was not provided.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The route and method of exposure were reported and were suited to the test substance; oral gavage.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Hepatic/Liver: The test animal species, strain, sex, age were reported. initial body weights were reported beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.; Renal/Kidney: The test animal species, strain, sex, age were reported. Initial body weight was reported in the results beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.; Immune/Hematological: The test animal species, strain, sex, age were reported. Initial body weight was reported in the results beginning the day of administration (following 1 week of acclimation). The test animal was obtained from a commercial source (Charles River Laboratories). The test species and strain were an appropriate animal model for the evaluation of the specific outcomes of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations.
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The number of animals per study group is unclear, but at least 6 animals per group were included (based on the number of animals evaluated).
Domain 5: Outcome Assessment				

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Study Citation:	Kwack, S., Kim, K., Kim, H., Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. Journal of Toxicology and Environmental Health, Part A: Current Issues 72(21-22):1446-1454.
Health Outcome(s):	Hepatic/Liver; Renal/Kidney; Immune/Hematological;
Reported Health Effect(s):	Hepatic/Liver: Relative liver weight (no significant effect), biochemical serum analysis (no effect on any parameter); Renal/Kidney: Relative kidney weight (no effect), relative adrenal weight (no effect), serum chemistry (BUN, creatinine, NA, K, CL, Ca), urinalysis; Immune/Hematological: Relative spleen weight (no effect), relative thymus weight (no effect), hematology (no hematological parameters effected);
Duration:	Short-term (>1-30 days) 4-weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	697382

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: Organ weights measured at day 28; limited methodology details for the hematology, serum biochemistry, and urinalysis were reported. The outcome assessment methodology partially addressed the intended outcomes of interest (e.g., serum chemistry and organ weight evaluated in the absence of histology).
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Medium	All Outcomes: 6/males/group were sampled; it is unclear how many total animals were in each exposure group
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Not applicable for this study
	Metric 20:	Negative Control Response	Medium	All Outcomes: The biological responses of the negative control group for the endpoints reported (organ weight, hematology, serum biochemistry) were adequate; however, quantitative results for the negative control response was not reported for urinalysis measurements.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Although the study did not report all information to determine confounding factors, reported information did not identify differences among study groups; there was no significant differences in food consumption between treatment group and control. Water consumption was not measured/reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical methods were described and adequate for comparison
	Metric 24:	Reporting of Data	Medium	All Outcomes: Data for exposure-related findings were presented for some outcomes (organ weight, hematology, serum biochemistry) by exposure group with mean and standard deviation.However, quantitative results for urinalysis measurements were not reported; results were qualitatively noted with limited information.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Oishi, S., Hiraga, K. (1980). Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations. Toxicology and Applied Pharmacology 53(1):35-41.
Health Outcome(s):	Renal/Kidney; Nutritional/Metabolic; Reproductive/Developmental; Hepatic/Liver;
Reported Health Effect(s):	Renal/Kidney: absolute kidney weight, relative kidney weight, levels of Zn in kidneys; Nutritional/Metabolic: Bodyweight gain; Reproductive/Developmental: testicular testosterone levels, serum testosterone levels, dihydrotestosterone levels, levels of zinc in testes; Hepatic/Liver: absolute liver weight, relative liver weight, levels of Zn in liver;
Duration:	Short-term (>1-30 days) 7-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61572

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: o-Phthalic acid (phthalic acid) was identified as the test substance.
	Metric 2:	Test Substance Source	High	All Outcomes: Authors report test substance source (Tokyo Kasei Kogyo Company) and authors report analytical verification of test substance
	Metric 3:	Test Substance Purity	High	All Outcomes: Purity of the test substance was verified analytically via GC-LC and TLC. Purity reported as "greater than 98%.".
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	All Outcomes: Details regarding the negative control croup were not reported (i.e., if it is unclear whether the negative control was untreated vs. a vehicle control)
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive control not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: The study did not report how animals were allocated to study groups. Authors report, the rats, "were distributedamong eight groups and fed diets containing 2% of PA"
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Information on preparation and storage was not reported, but the omission of details are unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	Medium	All Outcomes: Details of exposure administration are incompletely reported, but the missing information is unlikely to have a substantial impact on results
	Metric 9:	Reporting of Doses/Concentrations	Low	All Outcomes: Although the study reports food consumption was measured daily. the study did not report food intake in a dietary study. No statements indicating palatability issues were included for groups exposed to phthalic acid.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Continuously in the diet.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	All Outcomes: Only one exposure group was used. Justification for this dose selection was not provided and no significant effects were observed.
	Metric 12:	Exposure Route and Method	Low	All Outcomes: Minimal details about the methods for dietary exposure were reported, resulting in some uncertainty about the true exposure parameters, and no feed analysis (i.e., of the chemical content in the feed) is provided. Although the duration of exposure was only 7 days, there is uncertainty regarding the impact that these limitations may have on the results.
Domain 4: Test Animals				

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Study Citation:	Oishi, S., Hiraga, K. (1980). Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations. Toxicology and Applied Pharmacology 53(1):35-41.
Health Outcome(s):	Renal/Kidney; Nutritional/Metabolic; Reproductive/Developmental; Hepatic/Liver;
Reported Health Effect(s):	Renal/Kidney: absolute kidney weight, relative kidney weight, levels of Zn in kidneys; Nutritional/Metabolic: Bodyweight gain; Reproductive/Developmental: testicular testosterone levels, serum testosterone levels, dihydrotestosterone levels, levels of zinc in testes; Hepatic/Liver: absolute liver weight, relative liver weight, levels of Zn in liver;
Duration:	Short-term (>1-30 days) 7-day; short-term
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61572

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: Species, strain, age, sex and source were reported. Initial body weights were measured but not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Most husbandry conditions were reported (e.g., temperature, humidity, diet) and were adequate and assumed to be the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: 10 animals/group (20 in controls) were used in each group based on the results in Figure 2. No additional details were provided.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: Bodyweight gain; absolute or relative weight of the testes, liver, or kidneys; testicular testosterone levels; serum testosterone, dihydrotestosterone levels; levels of zinc in testes, liver, or serum; levels of zinc in kidneys
	Metric 17:	Consistency of Outcome Assessment	Low	All Outcomes: Timing of each outcome assessment and details of the study protocol execution were not provided.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Reported information indicates the study used adequate sampling for the outcome(s) of interest. 10 animals/group (20 in controls) were used in each group based on the results in Figure 2.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding is not required for any of the endpoints evaluated.
	Metric 20:	Negative Control Response	High	All Outcomes: Data for the control group was provided and appeared appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Authors report measuring body weight and food consumption daily, and do not report changes related to phthalic acid exposure.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	Low	All Outcomes: Authors report that statistical analysis was performed by an analysis of variance followed by a post hoc Scheffe test at a significance level of $p < 0.05$.
	Metric 24:	Reporting of Data	Medium	All Outcomes: Data for most outcomes were reported quantitatively in figures or tables or qualitatively in the text reported in the text for all outcomes.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Rahmani, A., Soleimannejad, K., Ahmadi, Hafezi, H., M.R., Asadollahi, K., Khalighi, Z. (2015). Prenatal Exposure to Phthalic Acid Induces Increased Blood Pressure, Oxidative Stress, and Markers of Endothelial Dysfunction in Rat Offspring. Cardiovascular Toxicology 16(4):307-315.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Prenatal exposure effects on body weight, vascular function, oxidative stress and cardiac nitric oxide synthase activity in 3 month offspring
Duration:	Short-term (>1-30 days) 10 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	3071054

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	The source of test substance identified as Aldrich, Milwaukee, WI. Lot/Batch number was not reported.
	Metric 3:	Test Substance Purity	High	The purity of the test substance was reported as 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A negative control group was included and appropriate.
	Metric 5:	Positive Controls	N/A	Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Low	Pregnant rats were randomly categorized into groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Preparation and storage were adequately described.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was consistent across the study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Study reports dosage as percentage and mg/kg. The amount of food consumed is not reported is based on cited reference. It is not clear if the authors measured the amount of food the animals in this study ate or if the calculations are based on the previous study.
	Metric 10:	Exposure Frequency and Duration	High	Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	There were minor uncertainties with doses used; a NOAEL was not obtained.
	Metric 12:	Exposure Route and Method	High	The exposure route was appropriate (diet).
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Age of animals is not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Not all husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals treated per group was appropriate.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Methodology was appropriate for outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

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Study Citation:	Rahmani, A., Soleimannejad, K., Ahmadi, Hafezi, H., M.R., Asadollahi, K., Khalighi, Z. (2015). Prenatal Exposure to Phthalic Acid Induces Increased Blood Pressure, Oxidative Stress, and Markers of Endothelial Dysfunction in Rat Offspring. Cardiovascular Toxicology 16(4):307-315.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Prenatal exposure effects on body weight, vascular function, oxidative stress and cardiac nitric oxide synthase activity in 3 month offspring
Duration:	Short-term (>1-30 days) 10 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	3071054

Domain	Metric		Rating	Comments
	Metric 18:	Sampling Adequacy	Low	Details regarding sampling of outcomes were not reported for all outcomes.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	Medium	Most data was reported. Data on volume densities were not reported.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Sung, J. E., Kim, J. E., Go, J., Koh, E. K., Song, S. H., Lee, H. A., Hwang, D. Y. (2016). Age-related response of IL-4/Luc/CNS-1 transgenic mice to phthalic anhydride exposure. Archives of Biological Sciences 68(1):145-154.
Health Outcome(s):	Skin/Connective Tissue; Nutritional/Metabolic; Immune/Hematological;
Reported Health Effect(s):	Skin/Connective Tissue: Changes in ear color, ear vein and other morphological characteristics.Ear thickness was measured to determine degree of skin inflammation.; Nutritional/Metabolic: Body weight; Immune/Hematological: Spleen, mesenteric lymph node and thymus weight and in vivo IL-4 luciferase signal in these organs.Serum IgE, histology on ear tissue for presence of immune cell accumulation (mast cells)Western blot on ear tissue for IL-6 and VEGF presence;
Duration:	Short-term (>1-30 days) 2-week
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5179546

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Test substance was identified as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of test substance and lot/Batch number were not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A negative control group was included and appropriate.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Study states the animals were randomly divided into groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Preparation and storage were not adequately described; however, this is unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Exposure was consistent across the study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	All Outcomes: Body weights were not reported, therefore dose calculations were based on default values (EPA 1988).
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: Only one dose was studied. That dose did elicit a response, but a NOAEL was not able to be obtained.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The exposure route was appropriate (dermal).
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	All Outcomes: Sex of the animals studied was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Husbandry conditions were reported and adequate.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals treated per group was acceptable (n=4-5).
Domain 5: Outcome Assessment				

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Study Citation:	Sung, J. E., Kim, J. E., Go, J., Koh, E. K., Song, S. H., Lee, H. A., Hwang, D. Y. (2016). Age-related response of IL-4/Luc/CNS-1 transgenic mice to phthalic anhydride exposure. Archives of Biological Sciences 68(1):145-154.
Health Outcome(s):	Skin/Connective Tissue; Nutritional/Metabolic; Immune/Hematological;
Reported Health Effect(s):	Skin/Connective Tissue: Changes in ear color, ear vein and other morphological characteristics.Ear thickness was measured to determine degree of skin inflammation.; Nutritional/Metabolic: Body weight; Immune/Hematological: Spleen, mesenteric lymph node and thymus weight and in vivo IL-4 luciferase signal in these organs.Serum IgE, histology on ear tissue for presence of immune cell accumulation (mast cells)Western blot on ear tissue for IL-6 and VEGF presence;
Duration:	Short-term (>1-30 days) 2-week
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5179546

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	Skin/Connective Tissue: Outcome methodology was appropriate for outcome of interest.; Nutritional/Metabolic: Methodology was appropriate for outcome of interest.; Immune/Hematological: Methodology was appropriate for outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Low	All Outcomes: Details regarding sampling of outcomes were not reported for all outcomes.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	High	All Outcomes: A negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was described and appropriate.
	Metric 24:	Reporting of Data	High	All Outcomes: Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Sung, J. E., Kim, J. E., Go, J., Koh, E. K., Song, S. H., Lee, H. A., Hwang, D. Y. (2016). Age-related response of IL-4/Luc/CNS-1 transgenic mice to phthalic anhydride exposure. Archives of Biological Sciences 68(1):145-154.
Health Outcome(s):	Cardiovascular; Lung/Respiratory; Renal/Kidney; Endocrine;
Reported Health Effect(s):	Cardiovascular: Heart weight and in vivo IL-4 luciferase signal in heart; Lung/Respiratory: Lung weight and in vivo imaging for IL-4 luciferase signal in lung; Renal/Kidney: Kidney wt and in vivo IL-4 luciferase signal in kidney; Endocrine: Pancreas weight and in vivo IL-4 luciferase signal in pancreas;
Duration:	Short-term (>1-30 days) 2-week
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5179546

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Test substance was identified as phthalic anhydride.
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of test substance and lot/Batch number were not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A negative control group was included and appropriate.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive control was not required in this study.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Study states the animals were randomly divided into groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Preparation and storage were not adequately described; however, this is unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Exposure was consistent across the study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	All Outcomes: Body weights were not reported, therefore dose calculations were based on default values (EPA 1988).
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Exposure frequency and duration were reported and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: Only one dose was studied. That dose did elicit a response, but a NOAEL was not able to be obtained.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The exposure route was appropriate (dermal).
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	All Outcomes: Sex of the animals studied was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Husbandry conditions were reported and adequate.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals treated per group was acceptable (n=4-5).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: Only organ weight and luciferase activity measured.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Low	All Outcomes: Details regarding sampling of outcomes were not reported for all outcomes.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not necessary for this study.

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Study Citation:	Sung, J. E., Kim, J. E., Go, J., Koh, E. K., Song, S. H., Lee, H. A., Hwang, D. Y. (2016). Age-related response of IL-4/Luc/CNS-1 transgenic mice to phthalic anhydride exposure. Archives of Biological Sciences 68(1):145-154.
Health Outcome(s):	Cardiovascular; Lung/Respiratory; Renal/Kidney; Endocrine;
Reported Health Effect(s):	Cardiovascular: Heart weight and in vivo IL-4 luciferase signal in heart; Lung/Respiratory: Lung weight and in vivo imaging for IL-4 luciferase signal in lung; Renal/Kidney: Kidney wt and in vivo IL-4 luciferase signal in kidney; Endocrine: Pancreas weight and in vivo IL-4 luciferase signal in pancreas;
Duration:	Short-term (>1-30 days) 2-week
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5179546

Domain	Metric		Rating	Comments
	Metric 20:	Negative Control Response	High	All Outcomes: A negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Study did not report all information to determine confounding, reported information did not identify differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was described and appropriate.
	Metric 24:	Reporting of Data	High	All Outcomes: Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Biagnini, R. E., Bernstein, D. I., Gallagher, J. S., Moorman, W. J., Knecht, E. A., Smallwood, A. W., Bernstein, I. L. (1988). Immune-responses of cynomolgus monkeys to phthalic-anhydride. Journal of Allergy and Clinical Immunology 82(1):23-29.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: The sensitization reactions from phthalic anhydride +/- protein by measuring dermal Evans Blue staining after intracutaneous injections.
Duration:	Subchronic (>30-91 days) 10 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5180411

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was identified as phthalic anhydride. The CAS number and specific form were not reported. No additional information on the test substance characterization was provided.
	Metric 2:	Test Substance Source	High	The test substance was obtained from J.T. Baker Chemical Co. in Phillipsburg, NJ. The lot number was reported as 124142. Analytical verification of the test substance was not specified.
	Metric 3:	Test Substance Purity	High	The test substance was reported to be 100.0% pure.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The negative control groups were injected with either Monkey serum albumin (MSA) (group 3) or the vehicle, a solution of ethanol and saline (group 4). Intracutaneous injections of ethanol and saline, PBS, and MSA alone were also included in the skin tests.
	Metric 5:	Positive Controls	Medium	Intracutaneous injections of histamine were also used in the skin test. Results for histamine reactions were provided in the text.
	Metric 6:	Randomized Allocation of Animals	Medium	The study notes animals were randomly assigned to one of the 4 treatment groups (2 exposed and 2 control).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Solutions were prepared within 3/4 hour prior to use. Storage information was not described for this 10-week study.
	Metric 8:	Consistency of Exposure Administration	Low	Details of exposure administration are insufficiently reported. It is not reported if animals were injected/exposed on the same day of the week or at the same time of day.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Subcutaneous injection volumes and mol or concentrations that were used were reported for the 2 test groups. A range of test concentrations (indicating 10-fold dilutions were used) were provided for the intracutaneous injections that were administered every 2 weeks.
	Metric 10:	Exposure Frequency and Duration	Medium	Animals were subcutaneously injected with test solutions weekly for 10 weeks. Intracutaneous injections were administered before and every 2 weeks during the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only 2 exposure groups for the subcutaneous injections were used, one with phthalic anhydride and MSA (group 1) and the other with phthalic anhydride only (group 2). All animals were intracutaneously injected with various solutions of histamine, ethanol and saline, PBS, MSA, phthalic anhydride, and phthalic anhydride and MSA for skin testing. Dose group selections were not justified by study authors, but changes in skin reactions were observed in the phthalic anhydride-MSA group.

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Study Citation:	Biagnini, R. E., Bernstein, D. I., Gallagher, J. S., Moorman, W. J., Knecht, E. A., Smallwood, A. W., Bernstein, I. L. (1988). Immune-responses of cynomolgus monkeys to phthalic-anhydride. Journal of Allergy and Clinical Immunology 82(1):23-29.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: The sensitization reactions from phthalic anhydride +/- protein by measuring dermal Evans Blue staining after intracutaneous injections.
Duration:	Subchronic (>30-91 days) 10 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5180411

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were appropriate for this study. Steps were taken to be sure the phthalic anhydride was able to be solubilized in the vehicle.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Young adult male cynomolgus monkeys (Macaca fascicularis) were used in this study and were obtained from Primate Imports Corporation in Port Washington, NY. Starting bodyweights ranged between 4.5-6.0 kg. The exact age of monkeys at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Animals were fed standard chow daily and fresh fruit 3 times per week. Water was available ad libitum. Animals were maintained on a 12-hour light-dark cycle. Animals were also checked for tuberculosis and parasites. Temperature and humidity conditions were not provided.
	Metric 15:	Number of Animals per Group	Medium	4 animals per group were used in this study (4 total groups).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	The methods used to determine positive skin reactions was not described. A template was used to measure the size of positive skin reactions 30 minutes after the injection. No additional information was provided.
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited or not reported.
	Metric 18:	Sampling Adequacy	Low	It was not reported if all 4 monkeys from each group were evaluated every 2 weeks for the skin test. Only results for the phthalic anhydride-MSA group were provided in a table for all 4 monkeys in the group.
	Metric 19:	Blinding of Assessors	N/A	Blinding of assessors was not done for skin reaction/sensitization assessments, but the assay is quantitative so it is not likely to have a large effect on the outcomes.
	Metric 20:	Negative Control Response	Medium	The biological responses of the negative control groups were generally described in the text.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Bodyweight changes and food and water consumption were not reported throughout the study. Additionally, some monkeys were used in previous experiments testing bronchoprovocation with an aqueous cotton extract (4-44 months prior to current study), injected with V2O5-protein conjugate (12 months prior to current study), or inhalation of V2O5 dust (24 months prior to current study). However, the authors stated that control experiments were done and concluded not to affect the current study.

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Study Citation:	Biagnini, R. E., Bernstein, D. I., Gallagher, J. S., Moorman, W. J., Knecht, E. A., Smallwood, A. W., Bernstein, I. L. (1988). Immune-responses of cynomolgus monkeys to phthalic-anhydride. Journal of Allergy and Clinical Immunology 82(1):23-29.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: The sensitization reactions from phthalic anhydride +/- protein by measuring dermal Evans Blue staining after intracutaneous injections.
Duration:	Subchronic (>30-91 days) 10 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5180411

Domain	Metric		Rating	Comments
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	Animals were tested for tuberculosis and parasites prior to the study and were free from both. No information was provided for evaluations during the study for health outcomes unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	Low	Fisher's exact test and other nonparametric methods were used for the skin tests. Data reported were insufficient to conduce independent statistical analyses. The study had a low N, so the study was statistically underpowered.
	Metric 24:	Reporting of Data	Low	Table 2 presents tabulated data for the phthalic anhydride-MSA group, but other results were reported in the text. Some text was poorly legible in the copy of the paper.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Biagnini, R. E., Bernstein, D. I., Gallagher, J. S., Moorman, W. J., Knecht, E. A., Smallwood, A. W., Bernstein, I. L. (1988). Immune-responses of cynomolgus monkeys to phthalic-anhydride. Journal of Allergy and Clinical Immunology 82(1):23-29.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: The sensitization reactions from phthalic anhydride +/- protein by measuring serum IgE and IgG
Duration:	Subchronic (>30-91 days) 10 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5180411

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was identified as phthalic anhydride. The CAS number and specific form were not reported. No additional information on the test substance characterization was provided.
	Metric 2:	Test Substance Source	High	The test substance was obtained from J.T. Baker Chemical Co. in Phillipsburg, NJ. The lot number was reported as 124142. Analytical verification of the test substance was not specified.
	Metric 3:	Test Substance Purity	High	The test substance was reported to be 100.0% pure.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The negative control groups were injected with either Monkey serum albumin (MSA) (group 3) or the vehicle, a solution of ethanol and saline (group 4). Baseline measurements of IgE were also collected prior to the study.
	Metric 5:	Positive Controls	Medium	Histamine was tested for the skin reaction. Positive controls are not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	The study notes animals were randomly assigned to one of the 4 treatment groups (2 exposed and 2 control).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Solutions were prepared within 3/4 hour prior to use. Storage information was not described for this 10-week study.
	Metric 8:	Consistency of Exposure Administration	Low	Details of exposure administration are insufficiently reported. It is not reported if animals were injected/exposed on the same day of the week or at the same time of day.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Subcutaneous injection volumes and mol or concentrations that were used were reported for the 2 test groups. A range of test concentrations (indicating 10-fold dilutions were used) were provided for the intracutaneous injections that were administered every 2 weeks.
	Metric 10:	Exposure Frequency and Duration	Medium	Animals were subcutaneously injected with test solutions weekly for 10 weeks. Intracutaneous injections were administered before and every 2 weeks during the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only 2 exposure groups for the subcutaneous injections were used, one with phthalic anhydride and MSA (group 1) and the other with phthalic anhydride only (group 2). All animals were intracutaneously injected with various solutions of histamine, ethanol and saline, PBS, MSA, phthalic anhydride, and phthalic anhydride and MSA for skin testing. Dose group selections were not justified by study authors, but changes in IgG antibodies were observed.

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Study Citation:	Biagnini, R. E., Bernstein, D. I., Gallagher, J. S., Moorman, W. J., Knecht, E. A., Smallwood, A. W., Bernstein, I. L. (1988). Immune-responses of cynomolgus monkeys to phthalic-anhydride. Journal of Allergy and Clinical Immunology 82(1):23-29.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: The sensitization reactions from phthalic anhydride +/- protein by measuring serum IgE and IgG
Duration:	Subchronic (>30-91 days) 10 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5180411

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were appropriate for this study. Steps were taken to be sure the phthalic anhydride was able to be solubilized in the vehicle.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Young adult male cynomolgus monkeys (Macaca fascicularis) were used in this study and were obtained from Primate Imports Corporation in Port Washington, NY. Starting bodyweights ranged between 4.5-6.0 kg. The exact age of monkeys at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Animals were fed standard chow daily and fresh fruit 3 times per week. Water was available ad libitum. Animals were maintained on a 12-hour light-dark cycle. Animals were also checked for tuberculosis and parasites. Temperature and humidity conditions were not provided.
	Metric 15:	Number of Animals per Group	Medium	4 animals per group were used in this study (4 total groups). The number of animals per group were low based on statistical considerations, but common for monkey experiments.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	The methods used to determine IgE levels were described and IgG was assessed before and every 2 weeks during the study. Other immunogenicity endpoints evaluated was the skin reaction test using Evan Blue dye.
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited or not reported.
	Metric 18:	Sampling Adequacy	High	IgG levels in Figure 2 indicate 4 monkeys per group were evaluated.
	Metric 19:	Blinding of Assessors	N/A	Blinding of assessors was not done, but the test is objective for for IgG assessment.
	Metric 20:	Negative Control Response	Medium	Negative control responses and baseline IgG levels are also plotted on Figure 2. By week 10, the MSA only group IgG levels were higher than the ethanol-saline only and the phthalic acid only groups.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Bodyweight changes and food and water consumption were not reported throughout the study. Additionally, some monkeys were used in previous experiments testing bronchoprovocation with an aqueous cotton extract (4-44 months prior to current study), injected with V2O5-protein conjugate (12 months prior to current study), or inhalation of V2O5 dust (24 months prior to current study). However, the study authors stated that control dermal experiments excluded the effects on the current study.

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Study Citation:	Biagnini, R. E., Bernstein, D. I., Gallagher, J. S., Moorman, W. J., Knecht, E. A., Smallwood, A. W., Bernstein, I. L. (1988). Immune-responses of cynomolgus monkeys to phthalic-anhydride. Journal of Allergy and Clinical Immunology 82(1):23-29.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: The sensitization reactions from phthalic anhydride +/- protein by measuring serum IgE and IgG
Duration:	Subchronic (>30-91 days) 10 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5180411

Domain	Metric		Rating	Comments
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	Animals were tested for tuberculosis and parasites prior to the study and were free from both. No information was provided for evaluations during the study for health outcomes unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	High	Kruskal-Wallis and Mann-Whitney U tests were used to evaluate changes in IgG levels. An N of 4 is statistically low however.
	Metric 24:	Reporting of Data	Medium	Data for each exposure group was reported in Figure 2 and findings were mostly described in the text. But individual IgG levels were only provided for one group of monkeys (phthalic anhydride with MSA). A table of results with exact IgE levels was not provided.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Murakami, K., Nishiyama, K., Higuti, T. (1986). Toxicity of dibutyl phthalate and its metabolites in rats. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene) 41(4):775-781.
Health Outcome(s):	Hepatic/Liver; Reproductive/Developmental; Renal/Kidney;
Reported Health Effect(s):	Hepatic/Liver: Liver weight, Liver histology, serum biochemistry, enzyme activity; Reproductive/Developmental: Testicular wt and histology; Renal/Kidney: Kidney wt, kidney histology, serum biochemistry;
Duration:	Subchronic (>30-91 days) 34-36 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61568

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Test substance was identified as phthalic acid (PA).
	Metric 2:	Test Substance Source	Low	All Outcomes: The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A concurrent negative control group was included.
	Metric 5:	Positive Controls	N/A	All Outcomes: Positive control was not applicable for this study.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: The study did not report how animals were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Preparation and storage were not described, however given the characteristics of the test substance, this is unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	All Outcomes: Details of exposure were limited; however, this is unlikely to substantially impact results.
	Metric 9:	Reporting of Doses/Concentrations	Low	All Outcomes: Animals were fed a diet containing 0, 0.5 or 5% of the test substance. The study does not report food intake, body weight or age of the animals used. There is no evidence of palatability differences or body weight differences.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate. Rats were fed the diet every day for 34 or 36 days.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	All Outcomes: There was no effect on any outcome even at the highest dose. It is not clear if the highest dose was high enough.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The exposure route and method were appropriate.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	All Outcomes: The source of the animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	All Outcomes: Husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals treated per group was appropriate (n=5)
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: Outcome assessment methodology were appropriate for intended outcomes

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Study Citation:	Murakami, K., Nishiyama, K., Higuti, T. (1986). Toxicity of dibutyl phthalate and its metabolites in rats. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene) 41(4):775-781.
Health Outcome(s):	Hepatic/Liver; Reproductive/Developmental; Renal/Kidney;
Reported Health Effect(s):	Hepatic/Liver: Liver weight, Liver histology, serum biochemistry, enzyme activity; Reproductive/Developmental: Testicular wt and histology; Renal/Kidney: Kidney wt, kidney histology, serum biochemistry;
Duration:	Subchronic (>30-91 days) 34-36 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61568

Domain	Metric		Rating	Comments
	Metric 17:	Consistency of Outcome Assessment	Medium	All Outcomes: Details regarding outcome assessment were limited but unlikely to have a substantial impact on results.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Sampling was adequate.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	High	All Outcomes: Negative control group response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: Food and water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	Low	All Outcomes: Statical analysis was performed but not described adequately.
	Metric 24:	Reporting of Data	High	All Outcomes: Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Murakami, K., Nishiyama, K., Higuti, T. (1986). Toxicity of dibutyl phthalate and its metabolites in rats. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene) 41(4):775-781.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Subchronic (>30-91 days) 34-36 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61568

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as phthalic acid (PA).
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A concurrent negative control group was included.
	Metric 5:	Positive Controls	N/A	Positive control was not applicable for this study.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Preparation and storage were not described, however given the characteristics of the test substance, this is unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure were limited; however, this is unlikely to substantially impact results.
	Metric 9:	Reporting of Doses/Concentrations	Low	Animals were fed a diet containing 0, 0.5 or 5% of the test substance. The study does not report food intake, body weight or age of the animals used. There is no evidence of palatability differences or body weight differences.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration were appropriate. Rats were fed the diet every day for 34 or 36 days.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	There was no effect on any outcome even at the highest dose. It is not clear if the highest dose was high enough.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were appropriate.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The source of the animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals treated per group was appropriate (n=5)
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methodology were appropriate for intended outcomes
	Metric 17:	Consistency of Outcome Assessment	Medium	Details regarding outcome assessment were limited but unlikely to have a substantial impact on results.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate.

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Study Citation:	Murakami, K., Nishiyama, K., Higuti, T. (1986). Toxicity of dibutyl phthalate and its metabolites in rats. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene) 41(4):775-781.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Subchronic (>30-91 days) 34-36 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61568

Domain	Metric		Rating	Comments
	Metric 19:	Blinding of Assessors	N/A	Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	Low	The body weights of the negative control were not reported. Data for test group was presented as % of control.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Food and water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	Low	Statical analysis was performed but not described adequately.
	Metric 24:	Reporting of Data	High	Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Murakami, K., Nishiyama, K., Higuti, T. (1986). Toxicity of dibutyl phthalate and its metabolites in rats. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene) 41(4):775-781.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: Spleen wt
Duration:	Subchronic (>30-91 days) 34-36 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61568

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as phthalic acid (PA).
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A concurrent negative control group was included.
	Metric 5:	Positive Controls	N/A	Positive control was not applicable for this study.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Preparation and storage were not described, however given the characteristics of the test substance, this is unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure were limited; however, this is unlikely to substantially impact results.
	Metric 9:	Reporting of Doses/Concentrations	Low	Animals were fed a diet containing 0, 0.5 or 5% of the test substance. The study does not report food intake, body weight or age of the animals used. There is no evidence of palatability differences or body weight differences.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration were appropriate. Rats were fed the diet every day for 34 or 36 days.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	There was no effect on any outcome even at the highest dose. It is not clear if the highest dose was high enough.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were appropriate.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The source of the animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals treated per group was appropriate (n=5)
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Only organ weight was assessed.
	Metric 17:	Consistency of Outcome Assessment	Medium	Details regarding outcome assessment were limited but unlikely to have a substantial impact on results.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate.

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Study Citation:	Murakami, K., Nishiyama, K., Higuti, T. (1986). Toxicity of dibutyl phthalate and its metabolites in rats. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene) 41(4):775-781.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: Spleen wt
Duration:	Subchronic (>30-91 days) 34-36 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	61568

Domain	Metric		Rating	Comments
	Metric 19:	Blinding of Assessors	N/A	Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	High	Negative control group response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Food and water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	No information was provided to either to support or dismiss differences in groups in health outcomes or attrition.
	Metric 23:	Data Presentation and Analysis	Low	Statical analysis was performed but not described adequately.
	Metric 24:	Reporting of Data	High	Exposure related outcomes were reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; body weight gain
Duration:	Subchronic (>30-91 days) 7-week Subchronic feeding pre-study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Nominal doses only (as ppm in diet) were reported. Study did not report food intake in a dietary study. No statements indicating palatability issues were included.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet for 7 weeks
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose/concentration were appropriate
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; body weight gain
Duration:	Subchronic (>30-91 days) 7-week Subchronic feeding pre-study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	Number per group was low (n=5/sex/dose), although the purpose of the study was for preliminary/dose-range finding purposes.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	All animals were evaluated
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	N/A	Mean control values were not reported.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	Statistical analysis was not performed, animal numbers were sufficient to allow analysis to determine percent change only (no measures of variance)
	Metric 24:	Reporting of Data	Medium	Results were reported as means in the absence of measures of variance. Control values were not reported.

Additional Comments: Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Survival
Duration:	Subchronic (>30-91 days) 7-week Subchronic feeding pre-study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Nominal doses only (as ppm in diet) were reported. Study did not report food intake in a dietary study. No statements indicating palatability issues were included.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet for 7 weeks
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose/concentration were appropriate
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Survival
Duration:	Subchronic (>30-91 days) 7-week Subchronic feeding pre-study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	Number per group was low (n=5/sex/dose), although the purpose of the study was for preliminary/dose-range finding purposes.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	All animals were evaluated
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not performed, there was no mortality in any group including controls
	Metric 24:	Reporting of Data	Medium	Results were reported in text (no mortalities)

Additional Comments: Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Hepatic/Liver
Reported Health Effect(s):	Hepatic/Liver: Liver histopathology
Duration:	Subchronic (>30-91 days) 7-week Subchronic feeding pre-study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Nominal doses only (as ppm in diet) were reported. Study did not report food intake in a dietary study. No statements indicating palatability issues were included.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet for 7 weeks
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose/concentration were appropriate
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Hepatic/Liver
Reported Health Effect(s):	Hepatic/Liver: Liver histopathology
Duration:	Subchronic (>30-91 days) 7-week Subchronic feeding pre-study
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	Number per group was low (n=5/sex/dose), although the purpose of the study was for preliminary/dose-range finding purposes.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported
	Metric 17:	Consistency of Outcome Assessment	Medium	There was incomplete reporting of the outcome assessment protocol. It is not clear whether there was consistency between groups
	Metric 18:	Sampling Adequacy	Low	Details regarding sampling of outcomes were not reported
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	Low	The biological response of the negative control groups were not reported
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Uninformative	Statistical analysis was not performed, results from controls are not reported, and therefore independent analysis cannot be performed.
	Metric 24:	Reporting of Data	Low	Data for exposure-related findings were not shown for each study group, but results were described in the text; severity was not described.

Additional Comments: Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Cardiovascular; Thyroid; Renal/Kidney; Gastrointestinal; Immune/Hematological; Musculoskeletal; Endocrine system (Endocrine tissues); Lung/Respiratory; Ocular/Sensory; Skin/Connective Tissue; Cancer/Carcinogenesis;
Reported Health Effect(s):	Cardiovascular: Histopathology (heart); Thyroid: Histopathology; Renal/Kidney: Histopathology; Gastrointestinal: Histopathology; Immune/Hematological: Histopathology; Musculoskeletal: Histopathology; Endocrine system (Endocrine tissues): Histopathology; Lung/Respiratory: Histopathology; Ocular/Sensory: Histopathology; Skin/Connective Tissue: Histopathology; Cancer/Carcinogenesis: Tumors;
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	All Outcomes: A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	All Outcomes: Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Basal diet
	Metric 5:	Positive Controls	N/A	All Outcomes: Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	All Outcomes: Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	All Outcomes: Study did not report food intake in a dietary study. No statements indicating palatability issues were included. Nominal doses reported.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Continuously in the diet
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	All Outcomes: Study only used 2 dose groups, in addition to controls. It is unclear if the high dose was high enough as no significant effects for the outcome of interest were observed.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Cardiovascular; Thyroid; Renal/Kidney; Gastrointestinal; Immune/Hematological; Musculoskeletal; Endocrine system (Endocrine tissues); Lung/Respiratory; Ocular/Sensory; Skin/Connective Tissue; Cancer/Carcinogenesis;
Reported Health Effect(s):	Cardiovascular: Histopathology (heart); Thyroid: Histopathology; Renal/Kidney: Histopathology; Gastrointestinal: Histopathology; Immune/Hematological: Histopathology; Musculoskeletal: Histopathology; Endocrine system (Endocrine tissues): Histopathology; Lung/Respiratory: Histopathology; Ocular/Sensory: Histopathology; Skin/Connective Tissue: Histopathology; Cancer/Carcinogenesis: Tumors;
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Uninformative	All Outcomes: Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: Species, strain, source, age, sex, and ranges of starting body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	All Outcomes: 50/sex/treatment group; 20/sex controls is too low for a chronic study
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	High	All Outcomes: The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis methods were clearly described and appropriate
	Metric 24:	Reporting of Data	High	All Outcomes: Incidence data were clearly presented.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Cardiovascular; Thyroid; Renal/Kidney; Gastrointestinal; Immune/Hematological; Musculoskeletal; Endocrine system (Endocrine tissues); Lung/Respiratory; Ocular/Sensory; Skin/Connective Tissue; Cancer/Carcinogenesis;
Reported Health Effect(s):	Cardiovascular: Histopathology (heart); Thyroid: Histopathology; Renal/Kidney: Histopathology; Gastrointestinal: Histopathology; Immune/Hematological: Histopathology; Musculoskeletal: Histopathology; Endocrine system (Endocrine tissues): Histopathology; Lung/Respiratory: Histopathology; Ocular/Sensory: Histopathology; Skin/Connective Tissue: Histopathology; Cancer/Carcinogenesis: Tumors;
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric	Rating	Comments
Additional Comments:	Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.		

Overall Quality Determination Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; body weight gain
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Study did not report food intake in a dietary study. No statements indicating palatability issues were included. Nominal doses reported.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Study only used 2 dose groups, in addition to controls. It is unclear if the high dose was high enough as no significant effects for the outcome of interest were observed.
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight; body weight gain
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	50/sex/treatment group; 20/sex controls is too low for a chronic study
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis methods were clearly described and appropriate
	Metric 24:	Reporting of Data	Low	Data were presented graphically as growth curves without measures of variance.

Additional Comments: Some text relevant to the outcome of interest appears to be missing. Page 45/118 (marked as page 24 in the document) starts with the end of a sentence.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs, relevant histopathology for brain
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Study did not report food intake in a dietary study. No statements indicating palatability issues were included. Nominal doses reported.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Study only used 2 dose groups, in addition to controls. It is unclear if the high dose was high enough as no significant effects for the outcome of interest were observed.
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs, relevant histopathology for brain
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	50/sex/treatment group; 20/sex controls is too low for a chronic study
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis methods were clearly described and appropriate
	Metric 24:	Reporting of Data	Medium	Incidence data were clearly presented. Results of clinical observations were not reported.

Additional Comments: Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Survival
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Study did not report food intake in a dietary study. No statements indicating palatability issues were included. Nominal doses reported.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Study only used 2 dose groups, in addition to controls. It is unclear if the high dose was high enough as no significant effects for the outcome of interest were observed.
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Survival
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	50/sex/treatment group; 20/sex controls is too low for a chronic study
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	Medium	Mortality of control male rats occurred earlier than the mortality of treated animals.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis methods were clearly described and appropriate
	Metric 24:	Reporting of Data	Medium	Data were presented graphically as probability of survival. Statistical analysis was presented in text.

Additional Comments: Some text relevant to the outcome of interest appears to be missing. Page 45/118 (marked as page 24 in the document) starts with the end of a sentence.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Hepatic/Liver
Reported Health Effect(s):	Hepatic/Liver: Liver histopathology
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Study did not report food intake in a dietary study. No statements indicating palatability issues were included. Nominal doses reported.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Study only used 2 dose groups, in addition to controls. It is unclear if the high dose was high enough as no significant effects for the outcome of interest were observed.
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Hepatic/Liver
Reported Health Effect(s):	Hepatic/Liver: Liver histopathology
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Low	50/sex/treatment group; 20/sex controls is too low for a chronic study
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Liver histopathology was reported in the absence of organ weight and serum chemistry data
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis methods were clearly described and appropriate
	Metric 24:	Reporting of Data	High	Incidence data were clearly presented.

Additional Comments: Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.

Overall Quality Determination — Medium

Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Histopathology
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as Phthalic Anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	A commercial source was reported. Lot/batch number was not provided, but the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	Purity Reported as 98.8%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were assigned to cages on a weight basis for a given species and sex and there were specific ranges of weight requirements (e.g., male rats were required to weigh 90-105 g).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	Preparation and storage conditions were specified. Mixing for heterogeneity was performed. Testing indicated when stored at RT for 2 weeks, loss was 2.59% (372 ppm) per day; however, it was indicated that storage was at 5degrees c. Loss was possibly due to hydrolysis of phthalic anhydride to phthalic acid, but this was not analytically verified. It was not specified what the loss was at this temperature. Diets were mixed fresh every 1-1.5 weeks (not daily), and changed in hoppers at least 3 times per week. Therefore, test substance was at RT in hoppers for approximately 2 days. The study indicated that the feed mixtures may have been unstable under the conditions of use (pg. 55).
	Metric 8:	Consistency of Exposure Administration	High	Animals from all groups were consistently treated and provided diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	Study did not report food intake in a dietary study. No statements indicating palatability issues were included.
	Metric 10:	Exposure Frequency and Duration	High	Continuously in the diet
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Study only used 2 dose groups, in addition to controls. It is unclear if the high dose was high enough as no significant effects for the outcome of interest were observed.
	Metric 12:	Exposure Route and Method	Uninformative	Parent compound loss from hydrolysis in diet is expected, and further uncertainty is raised by statement on page 55. Phthalic anhydride is expected to react with peptides in feed, which may further impacted the received doses. Preparation of food daily could have helped resolve stability issues (fresh diet was prepared every 1-1.5 wks). Gavage could have also corrected the identified issues.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, source, age, sex, and ranges of starting body weights were reported.

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Study Citation:	NCI, (1979). Bioassay of phthalic anhydride for possible carcinogenicity.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Histopathology
Duration:	Chronic (>91 days) 105 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63768; Linked HERO ID(s): 63768, 63763

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry conditions were completely reported and adequate; However, it was mentioned that control and dosed animals were housed in the same room as those being dosed with other compounds (2,4-diaminotoluene and 0-toluidine hydrochloride for rats) and 7 other compounds for mice; some of these chemicals are volatile.
	Metric 15:	Number of Animals per Group	Medium	50/sex/treatment group; 20/sex controls
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this outcome of interest.
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	It is unclear if there were confounding issues (food and water intake not reportred)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis methods were clearly described and appropriate
	Metric 24:	Reporting of Data	High	Incidence data were clearly presented.

Additional Comments: Due to: 1) The reported instability of the test substance in prepared food, 2) The lack of food and water intake measurements, or 3) lack of a statement reporting either consistent food consumption or no issues with palatability, and 4) Overall lack of observed effects, there is low confidence in the dosing in this study.

Overall Quality Determination — Medium

Study Citation:	Ema, M., Miyawaki, E., Harazono, A., Kawashima, K. (1997). Developmental toxicity evaluation of phthalic acid, one of the metabolites of phthalic acid esters, in rats. Toxicology Letters 2(3):109-115.
Health Outcome(s):	Reproductive/Developmental; Nutritional/Metabolic; Mortality;
Reported Health Effect(s):	Reproductive/Developmental: Reproductive parameters, numbers of live and dead fetuses, resorptions, fetal sex and bodyweight, external, internal and skeletal malformations.; Nutritional/Metabolic: Maternal food consumption and body weight; Mortality: Maternal mortality;
Duration:	Reproductive/Developmental GD 7-16
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	790543

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Phthalic acid. CASRN not provided, but structure was included.
	Metric 2:	Test Substance Source	High	All Outcomes: Commercial source reported; Batch and/or lot number were not included.
	Metric 3:	Test Substance Purity	High	All Outcomes: 99.5
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Basal diet
	Metric 5:	Positive Controls	N/A	All Outcomes: Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Study says animals were distributed ”on a random basis”, but does not specify the method of allocation. Body weights were similar across all groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Details of preparation but not storage were provided. No homogeneity or stability data.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: All animals had access to diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Doses in mg/kg/day were clearly reported and were based on animal BW and food intake data
	Metric 10:	Exposure Frequency and Duration	Low	All Outcomes: Authors provided appropriate justification for exposure GDs 7-16. However, this does not match current guidelines and could miss the critical developmental window for skeletal formation.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: The number of exposure groups and spacing were appropriate (went up to a maternally toxic dose)
	Metric 12:	Exposure Route and Method	Medium	All Outcomes: Typically gavage is preferred, but the authors provided appropriate Justification for exposure via diet.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: Species, strain, sex, life-stage, and initial body weights were provided. Source of test animals was not indicated.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The reported number of animals/group (n=11) is lower than the recommended number of ~20 females with implantation sites at necropsy.

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Study Citation:	Ema, M., Miyawaki, E., Harazono, A., Kawashima, K. (1997). Developmental toxicity evaluation of phthalic acid, one of the metabolites of phthalic acid esters, in rats. Toxicology Letters 2(3):109-115.
Health Outcome(s):	Reproductive/Developmental; Nutritional/Metabolic; Mortality;
Reported Health Effect(s):	Reproductive/Developmental: Reproductive parameters, numbers of live and dead fetuses, resorptions, fetal sex and bodyweight, external, internal and skeletal malformations.; Nutritional/Metabolic: Maternal food consumption and body weight; Mortality: Maternal mortality;
Duration:	Reproductive/Developmental GD 7-16
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	790543

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Reproductive/Developmental: The outcome assessment methodology addressed the intended outcomes of interest and the assessment methodology was sensitive and appropriate for the outcomes of interest.; Nutritional/Metabolic: The outcome assessment methodology addressed the intended outcomes of interest and the assessment methodology was sensitive and appropriate for the outcomes of interest.; Mortality: The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Reproductive/Developmental: Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.; Nutritional/Metabolic: Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups); Mortality: Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reproductive/Developmental: Reported information indicates the study used adequate sampling for the outcome(s) of interest.; Nutritional/Metabolic: Reported information indicates the study used adequate sampling for the outcome(s) of interest; Mortality: All animals were evlauated
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Not necessary for the outcome of interest
	Metric 20:	Negative Control Response	High	All Outcomes: The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: Food intake was considerably less in treated animals, particularly in the higher dose groups, which resulted in associated decreases in body weight.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: Postimplantation loss in the control group was high and may require comparison to historical control data for the strain.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical methods (including any calculations or data transformations) were clearly described or had only minor omissions and were appropriate for the dataset(s).
	Metric 24:	Reporting of Data	Medium	All Outcomes: Data for exposure-related findings were presented for all outcomes by exposure group and sex (if applicable) with quantal and/or continuous presentation. Missing standard deviations and important measurements, such as liter weight, may have some impact on analysis.

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Study Citation:	Ema, M., Miyawaki, E., Harazono, A., Kawashima, K. (1997). Developmental toxicity evaluation of phthalic acid, one of the metabolites of phthalic acid esters, in rats. Toxicology Letters 2(3):109-115.
Health Outcome(s):	Reproductive/Developmental; Nutritional/Metabolic; Mortality;
Reported Health Effect(s):	Reproductive/Developmental: Reproductive parameters, numbers of live and dead fetuses, resorptions, fetal sex and bodyweight, external, internal and skeletal malformations.; Nutritional/Metabolic: Maternal food consumption and body weight; Mortality: Maternal mortality;
Duration:	Reproductive/Developmental GD 7-16
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	790543

Domain	Metric	Rating	Comments
Additional Comments:	None		

Overall Quality Determination — High

Study Citation:	Ema, M., Miyawaki, E., Harazono, A., Kawashima, K. (1997). Developmental toxicity evaluation of phthalic acid, one of the metabolites of phthalic acid esters, in rats. Toxicology Letters 2(3):109-115.
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs of toxicity
Duration:	Reproductive/Developmental GD 7-16
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	790543

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Phthalic acid. CASRN not provided, but structure was included.
	Metric 2:	Test Substance Source	High	Commercial source reported; Batch and/or lot number were not included.
	Metric 3:	Test Substance Purity	High	99.5
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Basal diet
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Medium	Study says animals were distributed "on a random basis", but does not specify the method of allocation. Body weights were similar across all groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Details of preparation but not storage were provided. No homogeneity or stability data.
	Metric 8:	Consistency of Exposure Administration	High	All animals had access to diets ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	High	Doses in mg/kg/day were clearly reported and were based on animal BW and food intake data
	Metric 10:	Exposure Frequency and Duration	Low	Authors provided appropriate justification for exposure GDs 7-16. However, this does not match current guidelines and could miss the critical developmental window for skeletal formation.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and spacing were appropriate (went up to a maternally toxic dose)
	Metric 12:	Exposure Route and Method	Medium	Typically gavage is preferred, but the authors provided appropriate Justification for exposure via diet.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	Species, strain, sex, life-stage, and initial body weights were provided. Source of test animals was not indicated.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number of Animals per Group	Low	The reported number of animals/group (n=11) is lower than the recommended number of ~20 females with implantation sites at necropsy.
Domain 5: Outcome Assessment				

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Study Citation:	Ema, M., Miyawaki, E., Harazono, A., Kawashima, K. (1997). Developmental toxicity evaluation of phthalic acid, one of the metabolites of phthalic acid esters, in rats. Toxicology Letters 2(3):109-115.
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Clinical signs of toxicity
Duration:	Reproductive/Developmental GD 7-16
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	790543

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Medium	No details of the observation protocol were included.
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported.
	Metric 18:	Sampling Adequacy	Medium	Details regarding sampling of outcomes were not reported
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the outcome of interest
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Food intake was considerably less in treated animals, particularly in the higher dose groups, which resulted in associated decreases in body weight.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	Postimplantation loss in the control group was high and may require comparison to historical control data for the strain.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods (including any calculations or data transformations) were clearly described or had only minor omissions and were appropriate for the dataset(s).
	Metric 24:	Reporting of Data	Medium	Data for exposure-related findings were presented for all outcomes by exposure group and sex (if applicable) with quantal and/or continuous presentation. Missing standard deviations and important measurements, such as liter weight, may have some impact on analysis.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Fabro, S., Shull, G., Brown, N. A. (1982). The relative teratogenic index and teratogenic potency: proposed components of developmental toxicity risk assessment. Teratogenesis, Carcinogenesis, and Mutagenesis 2(1):61-76.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Teratogenicity index calculated (specific outcomes observed for phthalic anhydride were not reported)
Duration:	Reproductive/Developmental 3 days (GD 8-10)
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63760

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance is identified as phthalic anhydride
	Metric 2:	Test Substance Source	High	Test substance was obtained from Eastman Kodak Company (Rochester, NY)
	Metric 3:	Test Substance Purity	High	Test substances was determined to have purity >98%
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A negative control group receiving vehicle only was included
	Metric 5:	Positive Controls	N/A	Not necessary for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	PAD was prepared as a suspension in 0.5% (w/v) carboxymethyl cellulose solution immediately prior to injection to minimize hydrolysis. PAD hydrolyses to phthalic acid with a half-life on the order of seconds to minutes. Given the rapid rate of hydrolysis, there is concern that the chosen vehicle was not appropriate for PAD.
	Metric 8:	Consistency of Exposure Administration	High	Dams were treated with daily ip injections (0.01 ml/gm BW) on pregnancy days 8-10
	Metric 9:	Reporting of Doses/Concentrations	Uninformative	Study authors state that an average of 6 dose groups per compound (study reports 8 compounds tested) were tested and that the highest dose level for teratology studies was normally within the 95% confidence limits of the calculated LD01 concentration. The tested doses of phthalic anhydride were not reported.
	Metric 10:	Exposure Frequency and Duration	Uninformative	Dams were dosed on GDs 8-10. Current OECD 414 standards require dosing to be conducted throughout organogenesis and late gestation, typically up until the day before caesarean section, or as close as feasible. Limiting dosing to GDs 8-10 is inadequate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Uninformative	Study authors state that an average of 6 dose groups per compound (study reports 8 compounds tested) were tested and that the highest dose level for teratology studies was normally within the 95% confidence limits of the calculated LD01 concentration. However, the exact number of dose groups for phthalic anhydride were not reported (nor were the precise doses administered).
	Metric 12:	Exposure Route and Method	Medium	The test substance was administered via ip injection. The relevance of this route of exposure to humans is questionable.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Female CD-1 mice (Charles River Laboratories, Wilmington, MA) were used throughout the study. Age and starting body weight were not reported.

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Study Citation:	Fabro, S., Shull, G., Brown, N. A. (1982). The relative teratogenic index and teratogenic potency: proposed components of developmental toxicity risk assessment. Teratogenesis, Carcinogenesis, and Mutagenesis 2(1):61-76.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Teratogenicity index calculated (specific outcomes observed for phthalic anhydride were not reported)
Duration:	Reproductive/Developmental 3 days (GD 8-10)
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	63760

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Study authors state that mice were housed in climate-controlled conditions (20°C, 50% humidity, 0600-1800 hr light cycle) with free access to water and NIH 31 chow.
	Metric 15:	Number of Animals per Group	Medium	Authors state that at least 10 dams were included per dose group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Authors state that Animals were sacrificed on Day 18, the uteri were removed, and the numbers and locations of live, dead, and resorbing conceptuses were recorded. All live fetuses wereexamined for grossly observable malformations, weighed, and then dissected with the aid of a stereomicroscope to evaluate visceral defects and to designate gender 1121. Heads and skeletons were examined for abnormalities. Study authors did not include the following in their examination: undescended testes, extralung lobe, and several minor skeletal variants (cerival ribs, extra thoracic vertebra with pair of ribs), which reduces the sensitivity of their outcome analysis.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling.
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the outcome(s) being evaluated.
	Metric 20:	Negative Control Response	Low	The biological response of the negative control groups were not reported
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Confounding variables, such as body weight, food/water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods were clearly described and were appropriate for the dataset(s).
	Metric 24:	Reporting of Data	Uninformative	Data for individual observed malformations were not presented. Instead, the overall incidence of malformations in fetuses was presented as the malformation rate above the background rate and a teratogenic index was calculated.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Amoco, (1988). Letter from Amoco Corp to USEPA stating that the results of the report study on phthalic anhydride will be forwarded later.
Health Outcome(s):	Sensitization; Lung/Respiratory;
Reported Health Effect(s):	Sensitization: Respiratory sensitization (as determined by hemorrhagic foci) was observed in male and female rats.; Lung/Respiratory: Lung weight; lung volume.Additional information available in the associated reference (HEROID 12980190) provides additional information such as mild parabronchial lymphoid hyperplasia, alveolar hemorrhage, and perivascular acute and chronic inflammation.;
Duration:	Other (specify) respiratory sensitization
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5160442; Linked HERO ID(s): 12980190, 5160442

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Identified as phthalic anhydride; CASRN was provided.
	Metric 2:	Test Substance Source	High	All Outcomes: The source was not reportedUpdate: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the test substance source is Aldrich Chemical Company (Lot No. 00103DL).
	Metric 3:	Test Substance Purity	Uninformative	All Outcomes: Purity not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Details of the control group (air-only vs. untreated) was not specified.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the reports of the control group are identified.
	Metric 5:	Positive Controls	N/A	All Outcomes: Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: Animal allocation was not reported
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Preparation and storage were not reported.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the "test substance was ground to respirable-size particles using a mortar and pestle." Then these particles are aerosolized in a TSI Fluidized Bed Aerosol generator and pumped into the inhalation chamber for the rats.
	Metric 8:	Consistency of Exposure Administration	High	All Outcomes: Administration details were not reported.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as knowing that the experimental design was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: It was not specified whether exposure was to vapors. There was no indication exposures were verified analytically.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as that the exposure was to aerosol. Time weighted averages and actual concentrations the rats were exposed to were 525 ug/m3 based off of the range of exposure was 404 to 746 ug/m3. The lowest and highest is beyond the 20% deviation standard mentioned above.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Frequency and duration appeared to be appropriate for the purposes of the study.

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Study Citation:	Amoco, (1988). Letter from Amoco Corp to USEPA stating that the results of the report study on phthalic anhydride will be forwarded later.
Health Outcome(s):	Sensitization; Lung/Respiratory;
Reported Health Effect(s):	Sensitization: Respiratory sensitization (as determined by hemorrhagic foci) was observed in male and female rats.; Lung/Respiratory: Lung weight; lung volume.Additional information available in the associated reference (HEROID 12980190) provides additional information such as mild parabronchial lymphoid hyperplasia, alveolar hemorrhage, and perivascular acute and chronic inflammation.;
Duration:	Other (specify) respiratory sensitization
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5160442; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: No justifications for the number of exposure groups or concentration used was provided. The study cannot be used to identify a dose response. The concentration elicited a positive response.
	Metric 12:	Exposure Route and Method	Medium	All Outcomes: no description of the inhalation chamber was provided.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the study reports use of an inhalation chamber that the aerosolized Phthalic Anhydride was pumped into. No other descriptions of the inhalation chamber or the air changes were reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: No details of the test animal, other than species (rat) were provided.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as reporting that these are male and female sprague-dawley rats used. Rat body weights were measured before the experiments for "general well-being," but the untreated controls were not fasted in a cage so authors could not compare the body weights.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	All Outcomes: Animal husbandry details were not provided.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals/group was acceptable
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: Details of the outcome assessment methodology were poorly described but seemed appropriate for the purposes of the study.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as endpoints measured included serum antibody formation, and lung histopathology.
	Metric 17:	Consistency of Outcome Assessment	Medium	All Outcomes: There was limited reporting of outcome assessment methodology, but with the information provided it appears outcomes were assessed consistently across study groups.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the outcomes were reported at the end of the study and the groups were assessed consistently.
	Metric 18:	Sampling Adequacy	Medium	All Outcomes: The number of animals used for the generation of the data provided was not explicitly stated. It is assumed that all of the animals were evaluated.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Not necessary for the study type.
	Metric 20:	Negative Control Response	High	All Outcomes: The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).

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Study Citation:	Amoco, (1988). Letter from Amoco Corp to USEPA stating that the results of the report study on phthalic anhydride will be forwarded later.
Health Outcome(s):	Sensitization; Lung/Respiratory;
Reported Health Effect(s):	Sensitization: Respiratory sensitization (as determined by hemorrhagic foci) was observed in male and female rats.; Lung/Respiratory: Lung weight; lung volume.Additional information available in the associated reference (HEROID 12980190) provides additional information such as mild parabronchial lymphoid hyperplasia, alveolar hemorrhage, and perivascular acute and chronic inflammation.;
Duration:	Other (specify) respiratory sensitization
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5160442; Linked HERO ID(s): 12980190, 5160442

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as reporting differences and variables in the study.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	All Outcomes: Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as this study reported information that identified differences between experimental groups and their controls.
	Metric 23: Data Presentation and Analysis	Uninformative	All Outcomes: Statistical analyses were not performed, and because the number of animals or lungs examined were not provided statistical analyses can not be independently performed.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the number of animals examined is 10 males and 10 females for PA treatment and 10 males and 10 females were used for control group, so n=10/group.
	Metric 24: Reporting of Data	Medium	All Outcomes: Data for foci were adequately reported, however the number of animals (n) was not provided.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as an n=10/group.

Additional Comments: Data were presented in a letter and is not a full study report. It was indicated that the results reported were preliminary and based on verbal reports from laboratory personnel. This data is considered unreliable.Update: Additional information available in the associated reference (HEROID 12980190) provides additional information such as the full study report with results. This data is reliable to look at. However, even with the study report, there is a lack of clear information regarding the animal husbandry conditions and the wide range of concentration aerosolized. Additionally, no purity information was reported.

Overall Quality Determination — Uninformative

Study Citation:	Bae, C. J., Lee, J. W., Shim, S. B., Jee, S. W., Lee, S. H., Woo, J. M., Lee, C. K., Hwang, D. Y. (2011). GATA binding protein 3 overexpression and suppression significantly contribute to the regulation of allergic skin inflammation. International Journal of Molecular Medicine 28(2):171-179.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Ear thickness and histopathology; serum IgE, G1, G2a and G3; cytokine levels in lysate from ear and thymus; myeloperoxidase (MPO) activity in ear skin homogenate
Duration:	Other (specify) Tg animals: 3 x per week for 3 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177984

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported (Sigma-Aldrich). Certificates of analysis are available through the source website.
	Metric 3:	Test Substance Purity	Medium	The test substance purity was not reported; however, Sigma-Aldrich's website reports the purity as ≥99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to a vehicle control.
	Metric 5:	Positive Controls	N/A	A positive control was not required for this study type. The test substance was selected because it is a known inducer of allergic skin inflammation.
	Metric 6:	Randomized Allocation of Animals	Medium	Mice were randomly assigned to study groups; the method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Details of test substance preparation were limited. Test substance was prepared in 4:1 acetone: olive oil with a final concentration of 1%, 5% or 10%. The frequency of preparation was not reported. Storage conditions were not provided.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study animals. The test substance was applied to the dorsum of the ears 3 days/week for 3 weeks. The volume applied (50 µL) was reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal amount of test substance applied was reported. Animals were administered a nominal dose of 50 µl onto each ear. Body weight was not reported, so a mg/kg dose cannot be estimated.
	Metric 10:	Exposure Frequency and Duration	High	Test animals were exposed via dermal application to the dorsum of the ears three days a week for 3 weeks.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	3 dose groups and a control were included. The doses were the same as those used to treat WT mice. A dose-response was observed, although a NOAEL for Tg mice was not obtained.
	Metric 12:	Exposure Route and Method	High	Animals were dermally exposed to 50ul of solution on the dorsal side of the ears, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				

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Study Citation:	Bae, C. J., Lee, J. W., Shim, S. B., Jee, S. W., Lee, S. H., Woo, J. M., Lee, C. K., Hwang, D. Y. (2011). GATA binding protein 3 overexpression and suppression significantly contribute to the regulation of allergic skin inflammation. International Journal of Molecular Medicine 28(2):171-179.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Ear thickness and histopathology; serum IgE, G1, G2a and G3; cytokine levels in lysate from ear and thymus; myeloperoxidase (MPO) activity in ear skin homogenate
Duration:	Other (specify) Tg animals: 3 x per week for 3 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177984

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	Low	Transgenic mice overexpressing human GATA3 were generated. The age of the test animals was reported. Source, sex, and initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals exposed was not reported (n=6-8/group) and was appropriate.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	Details of the assessment protocol were reported and animals were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Low	The number of animals assessed was not reported in the figure legends. It is unclear if all animals were included in the analysis.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	The study authors report the use of a one-way ANOVA to determine differences between the test and control groups; however, significance was mostly reported for differences between the transgenic mice and the wildtype mice only. The number of mice in the figure legend was not reported; therefore, independent statistical analysis compared with controls is not possible for most endpoints.
	Metric 24:	Reporting of Data	Low	Histopathological findings were only reported for the middle dose group and the control. Dose levels for cytokine and chemokine data was not reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Bae, C. J., Lee, J. W., Shim, S. B., Jee, S. W., Lee, S. H., Woo, J. M., Lee, C. K., Hwang, D. Y. (2011). GATA binding protein 3 overexpression and suppression significantly contribute to the regulation of allergic skin inflammation. International Journal of Molecular Medicine 28(2):171-179.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Ear thickness and histopathology; serum IgE, G1, G2a and G3; cytokine levels in lysate from ear and thymus; myeloperoxidase (MPO) activity in ear skin homogenate
Duration:	Other (specify) WT animals: 3 x per week for 3 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177984

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported (Sigma-Aldrich). Certificates of analysis are available through the source website.
	Metric 3:	Test Substance Purity	Medium	The test substance purity was not reported; however, Sigma-Aldrich's website reports the purity as ≥99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to a vehicle control.
	Metric 5:	Positive Controls	N/A	A positive control was not required for this study type. The test substance was selected because it is a known inducer of allergic skin inflammation.
	Metric 6:	Randomized Allocation of Animals	Medium	Mice were randomly assigned to study groups; the method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Details of test substance preparation were limited. Test substance was prepared in 4:1 acetone: olive oil with a final concentration of 1%, 5% or 10%. The frequency of preparation was not reported. Storage conditions were not provided.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study animals. The test substance was applied to the dorsum of the ears 3 days/week for 3 weeks. The volume applied (50 µL) was reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal amount of test substance applied was reported. Animals were administered a nominal dose of 50 µl onto each ear. Body weight was not reported, so a mg/kg dose cannot be estimated.
	Metric 10:	Exposure Frequency and Duration	High	Test animals were exposed via dermal application to the dorsum of the ears three days a week for 3 weeks.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	3 dose groups and a control were included. A full range of responses were obtained (NOAEL and LOAEL).
	Metric 12:	Exposure Route and Method	High	Animals were dermally exposed to 50ul of solution on the dorsal side of the ears, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	Animals were referred to as "wildtype", exact strain is not reported. The age of the test animals was reported. Source, sex, and initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported.

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Study Citation:	Bae, C. J., Lee, J. W., Shim, S. B., Jee, S. W., Lee, S. H., Woo, J. M., Lee, C. K., Hwang, D. Y. (2011). GATA binding protein 3 overexpression and suppression significantly contribute to the regulation of allergic skin inflammation. International Journal of Molecular Medicine 28(2):171-179.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Ear thickness and histopathology; serum IgE, G1, G2a and G3; cytokine levels in lysate from ear and thymus; myeloperoxidase (MPO) activity in ear skin homogenate
Duration:	Other (specify) WT animals: 3 x per week for 3 weeks
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177984

Domain	Metric		Rating	Comments
	Metric 15:	Number of Animals per Group	Medium	The number of animals exposed was not reported (n=6-8/group) and was appropriate.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	Details of the assessment protocol were reported and animals were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Low	The number of animals assessed was not reported in the figure legends. It is unclear if all animals were included in the analysis.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	The study authors report the use of a one-way ANOVA to determine differences between the test and control groups; however, significance was mostly reported for differences between the transgenic mice and the wildtype mice only. The number of mice in the figure legend was not reported; therefore, independent statistical analysis compared with controls is not possible for most endpoints.
	Metric 24:	Reporting of Data	Low	Histopathological findings were only reported for the middle dose group and the control. Dose levels for cytokine and chemokine data was not reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Basketter, D. A., Scholes, E. W. (1992). Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology 30(1):65-69.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA test (lymphocyte proliferation) and Guinea-pig maximization test (erythema and edema at challenge site)
Duration:	Other (specify) LLNA- 3 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353562

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	The test substance purity was not reported; the study authors do report that the "vast majority of the compounds were more than 98% pure", but it is unclear if PA is one of those chemicals. Due to the date of the publication (1992), relying on the manufacturer's website for purity may not be accurate.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to a vehicle control.
	Metric 5:	Positive Controls	Low	No positive control was included, but treatment-related responses were observed. A positive control is recommended by OECD for the LLNA assay, but not required.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Details of test substance preparation are storage were not reported. Acetone-olive oil (4:1, v/v) was used as the vehicle; no other details are provided.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study animals. The test substance was applied to the dorsum of both ears for three consecutive days.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Concentrations were reported as a percent (%). They were not analytically verified. Animals were administered 25 µl onto each ear. Body weights were not reported, so a mg/kg dose cannot be estimated.
	Metric 10:	Exposure Frequency and Duration	High	Test animals were exposed via dermal application to the dorsum of both ears for three consecutive days.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Three concentrations (2.5%, 5%, and 10%) and a control group were included. Positive results were obtained at all doses, but a dose-response was not evident.
	Metric 12:	Exposure Route and Method	High	The assay was conducted as described by Basketter et al. 1991; this reference was not available to review. Exposure method details available in the current study were sufficient. Animals were dermally exposed on the dorsal side of both ears for 3 consecutive days, which is appropriate for the test substance and for the study type.

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Study Citation:	Basketter, D. A., Scholes, E. W. (1992). Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology 30(1):65-69.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA test (lymphocyte proliferation) and Guinea-pig maximization test (erythema and edema at challenge site)
Duration:	Other (specify) LLNA- 3 days
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353562

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The study used male and female CBA/Ca mice. It is not clear which sex was used for these studies, or if both sexes were studied. The age of the test animals was reported as a range (8-12 weeks). Initial body weights were not reported. The source of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals exposed (n=4/group) was reported and appropriate. OECD 429 guidelines recommend a minimum of 4 animals per group for the LLNA assay.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	The outcome assessment methodology for lymphocyte proliferation was appropriate. The study did not report body weight, which is recommended and may impact results (OECD 429).
	Metric 17:	Consistency of Outcome Assessment	High	Details of the LLNA assessment protocol were reported, and animals were consistently assessed across groups.
	Metric 18:	Sampling Adequacy	Low	The number of animals analyzed was not reported.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	Low	The biological response of the negative control group was not reported. The study calculated the ratio of test substance lymphocyte proliferation to control lymphocyte proliferation without showing control data.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis is not needed for this type of study.
	Metric 24:	Reporting of Data	Medium	The ratio of test substance lymphocyte proliferation to control lymphocyte proliferation is shown. Counts with variance were not shown.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Basketter, D. A., Scholes, E. W. (1992). Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology 30(1):65-69.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA test (lymphocyte proliferation) and Guinea-pig maximization test (erythema and edema at challenge site)
Duration:	Other (specify) Guinea-pig maximization test
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353562

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	The test substance purity was not reported; the study authors do report that the "vast majority of the compounds were more than 98% pure", but it is unclear if PA is one of those chemicals. Due to the date of the publication (1992), relying on the manufacturer's website for purity may not be accurate.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Uninformative	The study was purportedly carried out in a manner similar to that described by Magnusson and Klingman (1970); however, minimal details were provided, including whether or not proper negative controls were used.
	Metric 5:	Positive Controls	Low	A positive control was not included; however, the study tested 40 chemicals, many of which are well known to be skin sensitizers. Positive results were observed for several chemicals, including the COI.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Details of test substance preparation are storage were not reported. The induction injection vehicle was 0.9% NaCl with acetone if required. The vehicle for the topical applications was acetone-polyethene glycol 400 (70:30, v/v). No details on the adequacy of the vehicles were provided.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were sparsely reported in the current study, but were based on and similar to the methods described in Magnusson B. and Kligman A. M. (1970). The volumes applied or injected were not reported. Insufficient information is provided in the current study to assess the consistency of exposure administration.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Concentrations were reported as a percent (%). They were not analytically verified.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration of exposure were generally appropriate for the study type, but deviated from the current OECD TG 406. This is not expected to significantly impact the study results because the test gave the expected results.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	Preliminary irritation tests were done to determine suitable concentrations to use. The purpose of the study was not to determine a dose-response.
	Metric 12:	Exposure Route and Method	Medium	The exposure route was appropriate for the study type. GPMTs are routinely used to identify skin sensitizers. The methods were sparsely described in this study, but were reported to be similar to Magnusson and Kligman (1970); deviations were not specified. No details regarding the use of adjuvants were provided.

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Study Citation:	Basketter, D. A., Scholes, E. W. (1992). Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology 30(1):65-69.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA test (lymphocyte proliferation) and Guinea-pig maximization test (erythema and edema at challenge site)
Duration:	Other (specify) Guinea-pig maximization test
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5353562

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The study used albino Dunkin-Hartley guinea pigs weighing approximately 350 grams at the beginning of the study. Source, age, and sex of the animals were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Low	The number of animals/group is not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	The study states that test methods are based on Magnusson B. and Kligman A. M. (1970) Allergic Contact Dermatitis in the Guinea Pig. Edited by Charles C. Thomas. Springfield, IL., which was a well-recognized method at the time and sensitive to the outcomes of interest. No outcome assessment details were provided in the current study.
	Metric 17:	Consistency of Outcome Assessment	Medium	Insufficient information was provided to assess the consistency of outcome assessment.
	Metric 18:	Sampling Adequacy	Low	The number of animals analyzed was not reported.
	Metric 19:	Blinding of Assessors	Low	The study did not report blinding. Blind reading of responses is recommended (OECD TG 406).
	Metric 20:	Negative Control Response	N/A	It is unclear if a negative control group was included. Negative control responses should be reported.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis is not needed for this type of study.
	Metric 24:	Reporting of Data	Low	Scores of erythema and edema were not reported. Study reports percentage of animals with a positive response; the criteria for a positive response were not provided, and the severity of the responses was not described.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Blaikie, L., Morrow, T., Wilson, Hext, P., Hartop, P. J., Rattray, N. J., Woodcock, D. (1995). A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96(1):37-50.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization.
Duration:	Other (specify) One sensitization dose- dry air atmosphere
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177461

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as phthalic anhydride (PHA); CASRN not provided.
	Metric 2:	Test Substance Source	Low	A commercial source was reported. Lot and Batch numbers were not included and identity was not verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	The nature of the challenge controls was not clearly defined (air-only or untreated)
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Low	Allocation of animals was not specified.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Some details of atmosphere preparation are provided, but stability and analytical verification of concentrations were not clearly described.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner
	Metric 9:	Reporting of Doses/Concentrations	Low	Animals were exposed to an aerosol or particulate but MMAD/particle size data were not reported; however, effects were observed
	Metric 10:	Exposure Frequency and Duration	High	Exposure duration/frequency were appropriate for the purposes of the study
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose/concentration spacing were explicitly justified by study authors
	Metric 12:	Exposure Route and Method	Medium	Dermal induction and inhalation challenges appear to be common for testing for respiratory allergens. Issues with atmosphere generation were already addressed/downgraded in other metrics. Some details regarding airflow etc. were not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Strain, sex, source, and initial weight range were reported and appropriate. Age was not specified. Use of the species as a model system was justified.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animal husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Low	The number of animals vary per group, and low animal numbers decreased statistical power.

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Study Citation:	Blaikie, L., Morrow, T., Wilson, Hext, P., Hartop, P. J., Rattray, N. J., Woodcock, D. (1995). A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96(1):37-50.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization.
Duration:	Other (specify) One sensitization dose- dry air atmosphere
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177461

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the outcomes of interest
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.Note: Potential issues with atmosphere generation were addressed/downgraded in other metrics.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	Statistical analyses were not performed.
	Metric 24:	Reporting of Data	High	Data were adequately reported.

Additional Comments: Multiple experiments using different conditions were performed. This form is evaluating Laboratory 2; Experiment 2

Overall Quality Determination Medium

Study Citation:	Blaikie, L., Morrow, T., Wilson, Hext, P., Hartop, P. J., Rattray, N. J., Woodcock, D. (1995). A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96(1):37-50.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization.
Duration:	Other (specify) 3 sensitization doses; Dry-air and argon atmospheres
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177461

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as phthalic anhydride (PHA); CASRN not provided.
	Metric 2:	Test Substance Source	Low	A commercial source was reported. Lot and Batch numbers were not included and identity was not verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	The nature of the challenge controls was not clearly defined (air-only or untreated)
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Low	Allocation of animals was not specified.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Some details of atmosphere preparation are provided, but stability and analytical verification of concentrations were not clearly described.
	Metric 8:	Consistency of Exposure Administration	Low	The concentrations of the challenge exposures were reported as ranges. It is unclear whether challenge concentration exposures were consistent across the three induction groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	Animals were exposed to an aerosol or particulate but MMAD/particle size data were not reported; however, effects were observed. Changes concentrations were reported as a range only
	Metric 10:	Exposure Frequency and Duration	High	Exposure duration/frequency were appropriate for the purposes of the study
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose/concentration spacing were explicitly justified by study authors
	Metric 12:	Exposure Route and Method	Medium	Dermal induction and inhalation challenges appear to be common for testing for respiratory allergens. Issues with atmosphere generation were already addressed/downgraded in other metrics. Some details regarding airflow etc. were not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Strain, sex, source, and initial weight range were reported and appropriate. Age was not specified. Use of the species as a model system was justified.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animal husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Low	Number varies across groups, low numbers reduced statistical power.
Domain 5: Outcome Assessment				

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Study Citation:	Blaikie, L., Morrow, T., Wilson, Hext, P., Hartop, P. J., Rattray, N. J., Woodcock, D. (1995). A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96(1):37-50.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization.
Duration:	Other (specify) 3 sensitization doses; Dry-air and argon atmospheres
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177461

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the outcomes of interest
	Metric 20:	Negative Control Response	Medium	It is unclear why moderate to severe pulmonary responses were observed in 2/8 controls in one experiment.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.Note: Potential issues with atmosphere generation were addressed/downgraded in other metrics.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	Statistical analyses were not performed.
	Metric 24:	Reporting of Data	High	Data were adequately reported.

Additional Comments: Multiple experiments using different conditions were performed. This form is evaluating Laboratory 1; Experiments 1&2

Overall Quality Determination Medium

Study Citation:	Blaikie, L., Morrow, T., Wilson, Hext, P., Hartop, P. J., Rattray, N. J., Woodcock, D. (1995). A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96(1):37-50.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization.
Duration:	Other (specify) Single sensitization dose - Standard atmosphere generation
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177461

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as phthalic anhydride (PHA); CASRN not provided.
	Metric 2:	Test Substance Source	Low	A commercial source was reported. Lot and Batch numbers were not included and identity was not verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	The nature of the challenge controls was not clearly defined (air-only or untreated)
	Metric 5:	Positive Controls	N/A	Not necessary for the study type
	Metric 6:	Randomized Allocation of Animals	Low	Allocation of animals was not specified.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Uninformative	The study indicated that problems with test atmosphere preparation resulted in the lack of responsiveness observed. Specifically, oxidation of PHA particulates was reported to occur rapidly and therefore a different preparation method was proposed.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner
	Metric 9:	Reporting of Doses/Concentrations	Uninformative	Animals were exposed to an aerosol or particulate but MMAD/particle size data were not reported and no effects were observed at the highest dose. The study authors indicated problems with the dose accuracy due to issues with atmosphere generation.
	Metric 10:	Exposure Frequency and Duration	High	Exposure duration/frequency were appropriate for the purposes of the study
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and dose/concentration spacing were explicitly justified by study authors
	Metric 12:	Exposure Route and Method	Medium	Dermal induction and inhalation challenges appear to be common for testing for respiratory allergens. Issues with atmosphere generation were already addressed/downgraded in other metrics. Some details regarding airflow etc. were not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Strain, sex, source, and initial weight range were reported and appropriate. Age was not specified. Use of the species as a model system was justified.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animal husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Low	Number varies across groups, and low numbers reduced statistical power.
Domain 5: Outcome Assessment				

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Study Citation:	Blaikie, L., Morrow, T., Wilson, Hext, P., Hartop, P. J., Rattray, N. J., Woodcock, D. (1995). A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96(1):37-50.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization.
Duration:	Other (specify) Single sensitization dose - Standard atmosphere generation
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177461

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the outcomes of interest
	Metric 20:	Negative Control Response	High	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of outcomes of interest in the study).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.Note: Potential issues with atmosphere generation were addressed/downgraded in other metrics.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	Statistical analyses were not performed.
	Metric 24:	Reporting of Data	High	Data were adequately reported.

Additional Comments: Multiple experiments using different conditions were performed. This form is evaluating Laboratory 2; Experiment 1

Overall Quality Determination — Uninformative

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases the study included the following controls: acetone/acetone; acetone/PA; PA/acetone
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	In response to a workgroup request, the study conducted a comparison between a modified method with an increased frequency of applications (9 instead of 3 as specified in OECD 406) during the induction phase. The duration of exposure was appropriate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses was appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Details of outcome assessment were adequately reported. Animals were observed at least once a day during the study for clinical signs and mortality.
	Metric 17:	Consistency of Outcome Assessment	High	The frequency of observation was reported and consistent across groups.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not necessary for the outcome of interest.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not necessary (all animals survived).
	Metric 24:	Reporting of Data	High	The lack of death was inferred based on the data tables provided.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Clinical signs (Clinical signs)
Reported Health Effect(s):	Clinical signs (Clinical signs): Clinical signs
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases, the study included the following controls: acetone/acetone; acetone/PA; PA/acetone
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	In response to a workgroup request, the study conducted a comparison between a modified method with an increased frequency of applications (9 instead of 3 as specified in OECD 406) during the induction phase. The duration of exposure was appropriate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Clinical signs (Clinical signs)
Reported Health Effect(s):	Clinical signs (Clinical signs): Clinical signs
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Details of outcome assessment were adequately reported. Animals were observed at least once a day during the study for clinical signs and mortality.
	Metric 17:	Consistency of Outcome Assessment	High	The frequency of observation was reported and consistent across groups.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported but not necessary for outcome of interest.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not reported. Study authors report the few observed clinical signs were not related to the test substance but are observed spontaneously in this species. Therefore, animals were considered negative for clinical signs and statistics were not necessary.
	Metric 24:	Reporting of Data	Low	The study reported that "In the main test, dyspnea, hypoactivityand piloerection were observed in a few animals, but never in more than one animal per group." This is a general statement overall for a study that tested multiple chemicals; results for specific chemicals were not reported. However, this is considered to be a qualitative statement in the text of essentially negative findings.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weights
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases, the study included the following controls: acetone/acetone; acetone/PA; PA/acetone
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . ." there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	In response to a workgroup request, the study conducted a comparison between a modified method with an increased frequency of applications (9 instead of 3 as specified in OECD 406) during the induction phase. The duration of exposure was appropriate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weights
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Details of outcome assessment were adequately reported. Animals were weighed at the start of the study, on day 31 (at the time of a second challenge application), and on the last day of the study.
	Metric 17:	Consistency of Outcome Assessment	High	The frequency of observation was reported and consistent across groups.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported but not necessary for outcome of interest.
	Metric 20:	Negative Control Response	Medium	Insufficient information was provided to assess the appropriateness of the negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	Uninformative	Statistical analysis was not reported, and data for an independent analysis were not provided. The authors claimed that body weight gains were similar to those of controls.
	Metric 24:	Reporting of Data	High	Negative results were reported qualitatively in the text.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Skin sensitization
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; Batch no was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases, the study included the following controls: acetone/acetone; acetone/PA; PA/acetone
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes added to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	The study followed OECD 406 recommendations for frequency and duration of exposure. However, there are some concerns that the 6-hour duration adhesive applications of the substance are too infrequent to induce sensitization, especially with the unmodified version of the Buehler test. This study design may slightly bias the results towards the null, but there is consistency between groups. For the screening test, the authors did deviate from OECD TG by performing the second challenge exposure 14 days after the first (instead of after 7 days), but didn't see any problems with the validity of their assay, meaning this deficiency is likely minor.

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Skin sensitization
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The sensitization scoring system is described and is appropriate to assess the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	High	The authors confirm that assessors were unaware of treatment groups after the challenge phase, reducing the risk of observational bias with sensitization scoring.
	Metric 20:	Negative Control Response	High	The negative control responses were appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical methods are not described in the methods but were not required to determine sensitization.
	Metric 24:	Reporting of Data	Medium	The overall number of animals with positive sensitization scores at each timepoint was provided in the tables. No data on severity or irritation were provided.

Additional Comments: None

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Skin sensitization
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

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

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Other (specify) Skin sensitizaiton- 3-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases the study included the following controls: acetone/acetone; acetone/PA; PA/acetone. However, the control animals received a total of 9 dermal applications during the induction phase, whereas the test substance animals received 3, so conditions were not equal between the two groups.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	In response to a workgroup request, the study conducted a comparison between a modified method with an increased frequency of applications (9 instead of 3 as specified in OECD 406) during the induction phase. The duration of exposure was appropriate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Other (specify) Skin sensitizaiton- 3-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Details of outcome assessment were adequately reported. Animals were observed at least once a day during the study for clinical signs and mortality.
	Metric 17:	Consistency of Outcome Assessment	High	The frequency of observation was reported and consistent across groups.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not necessary for the outcome of interest.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not necessary (all animals survived).
	Metric 24:	Reporting of Data	High	The lack of death was inferred based on the data tables provided.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Clinical signs (Clinical signs)
Reported Health Effect(s):	Clinical signs (Clinical signs): Clinical signs
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases the study included the following controls: acetone/acetone; acetone/PA; PA/acetone. However, the control animals received a total of 9 dermal applications during the induction phase whereas the test substance animals received 3, so conditions were not equal between groups.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	In response to a workgroup request, the study conducted a comparison between a modified method with an increased frequency of applications (9 instead of 3 as specified in OECD 406) during the induction phase. The duration of exposure was appropriate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Clinical signs (Clinical signs)
Reported Health Effect(s):	Clinical signs (Clinical signs): Clinical signs
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Details of outcome assessment were adequately reported. Animals were observed at least once a day during the study for clinical signs and mortality.
	Metric 17:	Consistency of Outcome Assessment	High	The frequency of observation was reported and consistent across groups.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported but not necessary for outcome of interest.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not reported. Study authors report the few observed clinical signs were not related to the test substance but are observed spontaneously in this species. Therefore, animals were considered negative for clinical signs and statistics were not necessary.
	Metric 24:	Reporting of Data	Low	The study reported that "In the main test, dyspnea, hypoactivityand piloerection were observed in a few animals, but never in more than one animal per group." This is a general statement overall for a study that tested multiple chemicals; results for specific chemicals were not reported. However, this is considered to be a qualitative statement in the text of essentially negative findings.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weights
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	Proper vehicle controls were used for both the induction and challenge phases of the study. For the induction/challenge phases the study included the following controls: acetone/acetone; acetone/PA; PA/acetone. However, the control animals received a total of 9 dermal applications during the induction phase whereas the test substance animals received 3, so conditions were not equal between groups.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	In response to a workgroup request, the study conducted a comparison between a modified method with an increased frequency of applications (9 instead of 3 as specified in OECD 406) during the induction phase. The duration of exposure was appropriate.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weights
Duration:	Other (specify) Skin sensitizaiton- 9-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Details of outcome assessment were adequately reported. Animals were weighed at the start of the study, on day 31 (at the time of a second challenge application), and on the last day of the study.
	Metric 17:	Consistency of Outcome Assessment	High	The frequency of observation was reported and consistent across groups.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported but not necessary for outcome of interest.
	Metric 20:	Negative Control Response	Medium	Data were not provided to assess the appropriateness of the negative controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	Uninformative	Statistical analysis was not reported, and data for an independent analysis were not provided. The authors claimed that body weight gains were similar to those of controls.
	Metric 24:	Reporting of Data	High	Negative results were reported qualitatively in the text.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Skin sensitization
Duration:	Other (specify) Skin sensitizaiton- 3-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic anhydride (CAS No 85-44-9).
	Metric 2:	Test Substance Source	High	The test substance was supplied by Sigma; the batch number was provided.
	Metric 3:	Test Substance Purity	High	The purity is reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	For the induction/challenge phases the study included the following controls: acetone/acetone; acetone/PA; PA/acetone. Although the proper control types were included, they were not treated in the same manner as the treated groups. During induction, controls received 9 applications, whereas the treated group received 3.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type; but all tested chemicals were known sensitizers.
	Metric 6:	Randomized Allocation of Animals	Low	The authors do not state whether animals were randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage conditions of the test substance were not reported. The test substance solution was prepared fresh daily, but no details were provided regarding mixing or homogeneity. It is unlikely these deficiencies will substantially impact results. Tests were performed to determine a suitable vehicle and to determine the maximum solubility of the test substance in the vehicle (data not reported).
	Metric 8:	Consistency of Exposure Administration	Medium	The size of the applied filter paper (2.6 x 3.1 cm) was consistent. The volume added to the filter paper was not reported. The study reports the filter paper "was fully-loaded with test preparation (up to saturation) . . . " there may have been inconsistencies with the volumes add to the filter paper. Time of exposure was consistent (6 hours).
	Metric 9:	Reporting of Doses/Concentrations	Medium	The test substance solution was prepared fresh daily, but no steps were taken to ensure exposure concentrations were accurate. The percentage of the test substance in the vehicle was reported. Dosing in mg/kg cannot be determined because the volume applied was not specified. Initial body weights were reported as a range (300-500 g).
	Metric 10:	Exposure Frequency and Duration	High	The study followed OECD 406 recommendations for frequency and duration of exposure.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The number of exposure groups and doses were appropriate for the study's aim. Doses for the main test were based on preliminary and screening tests.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were suitable for the test substance.
Domain 4: Test Animals				

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Study Citation:	Botham, P., Urtizberea, M., Wiemann, C., Manciaux, X., Tilbury, L., Vohr, H. W., Allen, S., Carmichael, N. G., Jouffrey, De, S. (2005). A comparative study of the sensitivity of the 3-induction and 9-induction Buehler test procedures for assessing skin sensitisation potential. Food and Chemical Toxicology 43(1):65-75.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Skin sensitization
Duration:	Other (specify) Skin sensitizaiton- 3-day induction
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5177112

Domain		Metric	Rating	Comments
	Metric 13:	Test Animal Characteristics	High	The animal source, species, strain, sex, initial body weight, and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Housing conditions were fully reported.
	Metric 15:	Number of Animals per Group	Medium	OECD TG 406 recommends 20 total animals per group and 10 controls, which the study technically meets with 10/sex/group in the exposed groups and 5/sex/group in the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The sensitization scoring system is described and is appropriate to assess the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information provided, there is no indication of inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	Sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	High	The authors confirm that assessors were unaware of treatment groups after the challenge phase, reducing the risk of observational bias with sensitization scoring.
	Metric 20:	Negative Control Response	High	The negative control responses were appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Factors that could act as confounders were either controlled for in the study design or were measured during the study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	The study did not report any information to support or dismiss there were differences in animal attrition or health unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical methods are not described in the methods but were not required to determine sensitization.
	Metric 24:	Reporting of Data	Medium	The overall number of animals with positive sensitization scores at each time point was provided in the tables. No data on severity or irritation were provided.

Additional Comments: None

Overall Quality Determination — High

Study Citation:	Fukuyama, T., Tajima, Y., Ueda, H., Hayashi, K., Shutoh, Y., Harada, T., Kosaka, T. (2010). A method for measuring mouse respiratory allergic reaction to low-dose chemical exposure to allergens: an environmental chemical of uncertain allergenicity, a typical contact allergen and a non-sensitizing irritant. Toxicology Letters 195(1):35-43.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization. There were increased total IgE and IgG(1) levels; influx of eosinophils, neutrophils, chemokines and cytokines in BALF; increased surface antigen expression on B-cells in LNs; increased Th2 cytokine production in LNs; and increased respiratory allergy-related gene expression in both BALF and LNs.
Duration:	Other (specify) Dermal sensitization; intratracheal challenge
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1940789

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance identified as phthalic anhydride (PA); CASRN not provided.
	Metric 2:	Test Substance Source	Low	A commercial source was reported; batch/lot numbers were not provided.
	Metric 3:	Test Substance Purity	High	<99.5% pure
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Use of solvent negative controls were appropriate.
	Metric 5:	Positive Controls	N/A	The study did not include a specified positive control, however, several chemicals, known to be sensitizers were tested and positive results were observed.
	Metric 6:	Randomized Allocation of Animals	Low	Animals were randomly allocated, but the method of allocation was not specified.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Test solution preparation (solvents used) were reported, but storage was not described.
	Metric 8:	Consistency of Exposure Administration	High	The same volume was applied across groups
	Metric 9:	Reporting of Doses/Concentrations	Low	Doses can be calculated using Percentages of the test substance and volume applied. Animal body weights were not reported.
	Metric 10:	Exposure Frequency and Duration	High	Exposure frequencies and duration were appropriate for a sensitization study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The concentrations were carefully selected and well justified based on previous studies; therefore, the single exposure group was appropriate for the purposes of the study.
	Metric 12:	Exposure Route and Method	High	This study is primarily a methods validation study. Various chemicals with known irritation or sensitizing potential were evaluated to determine if the methods (previously established) was sensitive enough to distinguish between contact allergens and irritants.
Domain 4: Test Animals				

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Study Citation:	Fukuyama, T., Tajima, Y., Ueda, H., Hayashi, K., Shutoh, Y., Harada, T., Kosaka, T. (2010). A method for measuring mouse respiratory allergic reaction to low-dose chemical exposure to allergens: an environmental chemical of uncertain allergenicity, a typical contact allergen and a non-sensitizing irritant. Toxicology Letters 195(1):35-43.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization. There were increased total IgE and IgG(1) levels; influx of eosinophils, neutrophils, chemokines and cytokines in BALF; increased surface antigen expression on B-cells in LNs; increased Th2 cytokine production in LNs; and increased respiratory allergy-related gene expression in both BALF and LNs.
Duration:	Other (specify) Dermal sensitization; intratracheal challenge
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1940789

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	Medium	Species, strain, source, age, and sex were reported. BWs were not provided. The study authors indicated: The animal species used may affect the results of assays for respiratoryallergens. Limitations of mice are that their vasculatureis the actual target of the anaphylactic response, their bronchialmusculature is poorly developed and their small size can make itdifficult to detect pulmonary reactions associated with the elicitation of respiratory allergy," They justified the use of mouse due to their well-characterized immune system and use for MOA investigations. It was also stated that exhibit a number of similarities to humans in their response to respiratory allergens, including the development of IgE antibody and eosinophilic lung inflammation.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure
	Metric 15:	Number of Animals per Group	Medium	n=6/group
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome(s) of interest AND the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	Reported information indicates the study used adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	N/A	Not necessary for the study type
	Metric 20:	Negative Control Response	Medium	Data from the -/+ groups were not reported because the study authors indicated that according to preliminary studies, -/+ showed the same pattern as -/-, and therefore, only the -/- data were reported.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.

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Study Citation:	Fukuyama, T., Tajima, Y., Ueda, H., Hayashi, K., Shutoh, Y., Harada, T., Kosaka, T. (2010). A method for measuring mouse respiratory allergic reaction to low-dose chemical exposure to allergens: an environmental chemical of uncertain allergenicity, a typical contact allergen and a non-sensitizing irritant. Toxicology Letters 195(1):35-43.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization. There were increased total IgE and IgG(1) levels; influx of eosinophils, neutrophils, chemokines and cytokines in BALF; increased surface antigen expression on B-cells in LNs; increased Th2 cytokine production in LNs; and increased respiratory allergy-related gene expression in both BALF and LNs.
Duration:	Other (specify) Dermal sensitization; intratracheal challenge
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	1940789

Domain	Metric		Rating	Comments
	Metric 23:	Data Presentation and Analysis	High	Statistical methods (including any calculations or data transformations) were clearly described or had only minor omissions and were appropriate for the dataset(s).
	Metric 24:	Reporting of Data	High	Data from all groups were adequately reported.

Additional Comments: This study is primarily a methods validation study. Various chemicals with known irritation or sensitizing potential were evaluated to determine if the methods (previously established) was sensitive enough to distinguish between contact allergens and irritants.

Overall Quality Determination High

Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic anhydride. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Aldrich Chemical, Milwaukee, WI. Batch/lot number was identified (Lot No. 00103DL).
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Uninformative	The negative control group was untreated and not sham-exposed in a study where animals were exposed to a dust (aerosol). No challenge control was included. These are serious study design flaws that make it impossible to interpret the study results.
	Metric 5:	Positive Controls	N/A	A positive control was not included. Although there is no guideline for this study type specifying the need for a positive control, demonstration of proficiency in conducting respiratory sensitization studies yielding positive results would be appropriate.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of test chemical were not reported. The method and equipment used to aerosolize the test substance were briefly described.
	Metric 8:	Consistency of Exposure Administration	Low	Exposed and control animals were not consistently handled. For exposed animals, the concentrations in the sensitization phase were reported and ranged from 404 to 746 ug/m3 with a mean of 525 ug/m3. MMAD and GSD values were not reported for an aerosol. The chamber designs were not described; however, based on the information provided, animals were exposed in a dynamic chamber. Homogeneity of the test substance within the chamber was not assessed. The number of animals in the inhalation chamber was not reported; it is unclear whether there was a single or multiple chambers used in the study.
	Metric 9:	Reporting of Doses/Concentrations	Low	Both target and analytical concentrations were reported. A time-weighted average concentration was reported for the sensitization phase along with the range, and the challenge concentration was measured analytically. It is unclear why two different wavelengths were used for the UV/Vis Spectrophotometer to measure the same chemical. Particle size, MMAD, and GSD were not reported. A positive reaction was observed, but it is unclear if it was test-substance related.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	One concentration was studied. The authors do not provide justification for choosing concentration, but positive results were seen.

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Mortality
Reported Health Effect(s):	Mortality: Death
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	Whole-body inhalation chambers were used. Since the test substance was delivered as an aerosol, it is likely that the test substance was deposited on the fur. There is no indication that the animals were wiped down after exposure. The number of air changes/hour was not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, strain were reported. Starting body weights and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported except to note that the control animals were not fasted in their cage like the PA exposed group was during inhalation.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (10/sex/group) was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate and described adequately. Animals were observed daily for mortality and morbidity.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups. The frequency of observation was reported.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate and all animals were accounted for in data tables.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not necessary for outcomes of interest.
	Metric 20:	Negative Control Response	High	Negative control response was appropriate. No animals died.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, respiratory rates should be measured. Terminal body weights were reported, but not the weekly measured body weights or food intake. Exposed animals were fasted during each exposure period; however, control animals were not fasted.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Not necessary for mortality (no deaths occurred)
	Metric 24:	Reporting of Data	High	Data were fully reported.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization (serum IgG)
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic anhydride. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Aldrich Chemical, Milwaukee, WI. Batch/lot number was identified (Lot No. 00103DL).
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Uninformative	The negative control group was untreated and not sham-exposed in a study where animals were exposed to a dust (aersosol). No challenge control was included. These are serious study design flaws that make it impossible to interpret the study results.
	Metric 5:	Positive Controls	N/A	A positive control was not included. Although there is no guideline for this study type specifying the need for a positive control, demonstration of proficiency in conducting respiratory sensitization studies yielding positive results would be appropriate.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of test chemical were not reported. The method and equipment used to aerosolize the test substance were briefly described.
	Metric 8:	Consistency of Exposure Administration	Low	Exposed and control animals were not consistently handled. For exposed animals, the concentrations in the sensitization phase were reported and ranged from 404 to 746 ug/m3 with a mean of 525 ug/m3. MMAD and GSD values were not reported for an aerosol. The chamber designs were not described; however, based on the information provided, animals were exposed in a dynamic chamber. Homogeneity of the test substance within the chamber was not assessed. The number of animals in the inhalation chamber was not reported; it is unclear whether there was a single or multiple chambers used in the study.
	Metric 9:	Reporting of Doses/Concentrations	Low	Both target and analytical concentrations were reported. A time-weighted average concentration was reported for the sensitization phase along with the range, and the challenge concentration was measured analytically. It is unclear why two different wavelengths were used for the UV/Vis Spectrophotometer to measure the same chemical. Particle size, MMAD, and GSD were not reported. A positive reaction was observed, but it is unclear if it was test-substance related.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	One concentration was studied. The authors do not provide justification for choosing concentration, but positive results were seen.

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization (serum IgG)
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	Whole-body inhalation chambers were used. Given that the test substance was delivered as an aerosol, the substance was likely deposited on the fur. There is no indication that the animals were wiped down after exposure. The number of air changes/hour was not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, and strain were reported. Starting body weights and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported except to note that the control animals were not fasted in their cage, like the PA-exposed group was during inhalation.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (10/sex/group) was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methods were appropriate and sensitive to the outcome of interest. To assess respiratory sensitization, serum IgG levels were measured using ELISA, and lungs were examined for hemorrhagic foci, where animals with $\geq$10 foci/lung were assigned a positive response.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate, and all animals were accounted for in the data tables.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not necessary for outcomes of interest.
	Metric 20:	Negative Control Response	High	Negative control response was appropriate; although the negative control itself was not appropriate (see metric 4).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, respiratory rates should be measured. Terminal body weights were reported, but not the weekly measured body weights or food intake. Exposed animals were fasted during each exposure period; however, control animals were not fasted.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	Statistical analysis was performed; however, the methods were not described. Data is sufficiently provided for independent analysis.
	Metric 24:	Reporting of Data	High	Data were fully reported with individual animal data.
Additional Comments:	None			

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory sensitization (serum IgG)
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

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

Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Lung weight, lung volume, gross necropsy, histopathology
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic anhydride. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Aldrich Chemical, Milwaukee, WI. Batch/lot number was identified (Lot No. 00103DL).
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Uninformative	The negative control group was untreated and not sham-exposed in a study where animals were exposed to a dust (aersosol). No challenge control was included. These are serious study design flaws that make it impossible to interpret the study results.
	Metric 5:	Positive Controls	N/A	A positive control was not included. Although there is no guideline for this study type specifying the need for a positive control, demonstration of proficiency in conducting respiratory sensitization studies yielding positive results would be appropriate.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of test chemical were not reported. The method and equipment used to aerosolize the test substance were briefly described.
	Metric 8:	Consistency of Exposure Administration	Low	Exposed and control animals were not consistently handled. For exposed animals, the concentrations in the sensitization phase were reported and ranged from 404 to 746 ug/m3 with a mean of 525 ug/m3. MMAD and GSD values were not reported for an aerosol. The chamber designs were not described; however, based on the information provided, animals were exposed in a dynamic chamber. Homogeneity of the test substance within the chamber was not assessed. The number of animals in the inhalation chamber was not reported; it is unclear whether there was a single or multiple chambers used in the study.
	Metric 9:	Reporting of Doses/Concentrations	Low	Both target and analytical concentrations were reported. A time-weighted average concentration was reported for the sensitization phase along with the range, and the challenge concentration was measured analytically. It is unclear why two different wavelengths were used for the UV/Vis Spectrophotometer to measure the same chemical. Particle size, MMAD, and GSD were not reported. A positive reaction was observed, but it is unclear if it was test-substance related.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	One concentration was studied. The authors do not provide justification for choosing concentration, but positive results were seen.

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Lung weight, lung volume, gross necropsy, histopathology
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	Whole-body inhalation chambers were used. Given that the test substance was delivered as an aerosol, the substance was likely deposited on the fur. There is no indication that the animals were wiped down after exposure. The number of air changes/hour was not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, strain were reported. Starting body weights and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported except to note that the control animals were not fasted in their cage like the PA exposed group was during inhalation.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (10/sex/group) was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	Outcome assessment methodology partially addressed the outcome of interest. Histopathological examination on the lungs was done on only 1/10 females/group, and 2/10 males/group. Organ weights, lung volume and gross necropsy were assessed in all animals.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Medium	Histopathology was only conducted on 3 animals total (mixed sex for some groups, same sex for others). Lung weights and lung volume endpoints were measured for all animals.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not necessary for outcomes of interest.
	Metric 20:	Negative Control Response	High	The negative control response was appropriate, although the negative control used was not appropriate (see metric 4).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, respiratory rates should be measured. Terminal body weights were reported, but not the weekly measured body weights or food intake. Exposed animals were fasted during each exposure period; however, control animals were not fasted.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	Low	Statistical methods are not described, although statistical significance is indicated in data tables for some outcomes (i.e., lung weight, lung volume, lung foci). Statistical analysis was not performed for histopathology results because an insufficient number of animals were examined.

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Lung weight, lung volume, gross necropsy, histopathology
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric	Rating	Comments
	Metric 24: Reporting of Data	High	Data were fully reported with individual animal data. The severity of histopathological lesions was described.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic anhydride. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Aldrich Chemical, Milwaukee, WI. Batch/lot number was identified (Lot No. 00103DL).
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Uninformative	The negative control group was untreated and not sham-exposed in a study where animals were exposed to a dust (aerosol). No challenge control was included. These are serious study design flaws that make it impossible to interpret the study results.
	Metric 5:	Positive Controls	N/A	A positive control was not included. Although there is no guideline for this study type specifying the need for a positive control, demonstration of proficiency in conducting respiratory sensitization studies yielding positive results would be appropriate.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of test chemical were not reported. The method and equipment used to aerosolize the test substance were briefly described.
	Metric 8:	Consistency of Exposure Administration	Low	Exposed and control animals were not consistently handled. For exposed animals, the concentrations in the sensitization phase were reported and ranged from 404 to 746 ug/m3 with a mean of 525 ug/m3. MMAD and GSD values were not reported for an aerosol. The chamber designs were not described; however, based on the information provided, animals were exposed in a dynamic chamber. Homogeneity of the test substance within the chamber was not assessed. The number of animals in the inhalation chamber was not reported; it is unclear whether there was a single or multiple chambers used in the study.
	Metric 9:	Reporting of Doses/Concentrations	Low	Both target and analytical concentrations were reported. A time-weighted average concentration was reported for the sensitization phase along with the range, and the challenge concentration was measured analytically. It is unclear why two different wavelengths were used for the UV/Vis Spectrophotometer to measure the same chemical. Particle size, MMAD, and GSD were not reported. A positive reaction was observed, but it is unclear if it was test-substance related.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	One concentration was studied. The authors do not provide justification for choosing concentration, but positive results were seen.

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	Whole-body inhalation chambers were used. Given that the test substance was delivered as an aerosol, the substance was likely deposited on the fur. There is no indication that the animals were wiped down after exposure. The number of air changes/hour was not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, and strain were reported. Starting body weights and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported except to note that the control animals were not fasted in their cage, like the PA-exposed group was during inhalation.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (10/sex/group) was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Uninformative	Unlike the animals exposed to the test substance, animals in the control group were not fasted for the exposure time (6 hours/day, 5 days). The study authors state because of this, body weights cannot be compared and were included as a measure of the animal's well-being.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate and all animals were accounted for in data tables.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not necessary for outcomes of interest.
	Metric 20:	Negative Control Response	High	Negative control response was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The test substance is a respiratory irritant; therefore, respiratory rates should be measured. Terminal body weights were reported, but not the weekly measured body weights or food intake. Exposed animals were fasted during each exposure period; however, control animals were not fasted.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not performed because the controls were not an appropriate comparator.
	Metric 24:	Reporting of Data	Low	Only terminal body weights were reported.
Additional Comments:	None			

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Study Citation:	IIT Research Institute, (1996). Respiratory sensitization study of phthalic anhydride (PA): A research project [redacted].
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Other (specify) Respiratory sensitizaton
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	12980190; Linked HERO ID(s): 12980190, 5160442

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

Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Inhalation
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	65818

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic anhydride. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Aldrich Chemical, Milwaukee, WI. Batch/lot number was not identified.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study appropriately included an air-only (negative inhalation) control group and a GPSA-only control in the PCA test.
	Metric 5:	Positive Controls	N/A	Positive control was not included, but test chemical is a known sensitizer. Postive responses were obtained.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of test chemical were not reported. The method and equipment used to aerosolize the test substance were briefly described. Dust was produced by sonic disturbance of micronized dust in Pitt No 3 dust generator. The preparation of the chemical-protein conjugate is adequately reported.
	Metric 8:	Consistency of Exposure Administration	Low	The test substance was not consistently delivered. The study authors report the concentrations delivered as ranges (0.05 to 0.2 mg/m3 and 0.6 to 6 mg/m3) due to the day-to-day difficulty in controlling the dust levels. Atmospheres were between 65% to 80% respirable, and MMAD ranged from 5.8 to 9.8 um. Daily averages with variance were not reported.
	Metric 9:	Reporting of Doses/Concentrations	Low	Concentrations were measured by collecting chamber air onto glass-fiber filters, eluting the PA into acetonitrile, and measuring the optical density at 230 nm. The amount of PA was calculated from a PA standard curve. The mean mass diameter was between 5.8 and 9.8um. The dust was reported to be between 65-80% respirable (<10um) but a GSD or distribution data were not reported. The concentrations were reported as a range with no information on average or standard error. Animals were challenged with a PA:GPSA conjugate. A molar ratio of 25:1 was reported; the concentration of the conjugate was not provided.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	Two exposure groups were studied. Both a positive and negative response were seen in the high and low dose, respectively.

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Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Inhalation
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	65818

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	Animals were placed in plethysmographs and exposed as described in Karol et al. 1981). This citation was reviewed. The cited study indicates that animals were exposed head-only. The current study does not indicate whether animals were properly acclimated. The flow rate was not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, strain and starting body weights were reported. Age or life-stage was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Food and water were available ad libitum (except during inhalation exposure). No other husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (5 or 6/group) was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate and described adequately
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate and all animals were accounted for in data tables.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported, however outcomes assessed were not subjective in nature.
	Metric 20:	Negative Control Response	Medium	The air-only, PA-GPSA challenged negative control responses were appropriate. Elisa results for anti-GPSA alone (a negative control) were not reported.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Not all information was reported to determine confounding, reported information did not identify any differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods were reported and appropriate; or responses were reported as positive/negative.
	Metric 24:	Reporting of Data	Medium	Data were reported fully for most outcomes of interest. Serum antibody titers were reported as negative for the low concentration group in the text. Results for challenges with GPSA alone

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Subcutaneous
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	65818

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic anhydride. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Aldrich Chemical, Milwaukee, WI. Batch/lot number was not identified.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Appropriate controls were used for most of the tests included. A vehicle-only control group was included for the injection regimen. A GPSA alone (negative control) group was used for ACA, PCA, and ELISA tests. However, the intratracheal challenge was done with PA-GPSA only; a GPSA alone group was not included, but would have been appropriate.
	Metric 5:	Positive Controls	N/A	Positive control was not included, but test chemical is a known sensitizer. Postive responses were obtained.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of the test chemical were not reported. The test substance was dissolved in olive oil prior to injections; no details are provided. The lack of details is unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	The test substance was delivered subcutaneously 2 times/week for 4 weeks. The volume administered was consistent. The anatomical location where the test substance was delivered was not reported.
	Metric 9:	Reporting of Doses/Concentrations	Low	Doses were reported as a molar dose (400 uL of 6.7 x 10-5 M, 6.7 x10-4 M, or 6.7 x10-3 M) for ease of comparison with other chemicals tested. The volume administered is reported. Initial body weights were reported, but it is unclear how much weight the animals gained throughout the 28-day period; therefore, calculated doses in mg/kg-day based on initial body weights would likely not represent the actual doses. 100uL of the PA-GPSA conjugate (500 ug/mL) was delivered intratracheally.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Dosing injections were chosen based on initial range-finding studies using weight/volume doses of phthalic anhydride. Positive responses were seen at all dose levels. Testing a lower level may give more information and set a lower limit.
	Metric 12:	Exposure Route and Method	Medium	The exposure route and method were appropriate for the test chemical and the aim of the study. The injection site on the animals was not reported.
Domain 4: Test Animals				

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Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Subcutaneous
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	65818

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, strain and starting body weighs were reported. Age or life-stage were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Food and water were available ad libitum. No other husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (10/group) was appropriate. The control group included 30 animals (separate control groups were included for each chemical tested in the study).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Outcome assessment methodology was appropriate. Details on the PCA test were confusing and not clearly outlined.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information available, outcomes appear to assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate and all animals were accounted for in data tables.
	Metric 19:	Blinding of Assessors	Low	Blinding was not reported. For the intratracheal challenge, changes in respiratory rates were visually monitored for 10 minutes. The assessor was looking for the number of diaphragmatic contractions. This could be subjective. Other outcomes were not subjective in nature.
	Metric 20:	Negative Control Response	Low	The study did not report ACA test or PCA test, or ELISA results for the GPSA alone controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Not all information was reported to determine confounding, reported information did not identify any differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods were reported and appropriate; or responses were reported as positive/negative.
	Metric 24:	Reporting of Data	High	Data were reported fully for all outcomes of interest except for the GPSA controls (see Metric 20). Incidence data and mean +/- variance were reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Sarlo, K., Clark, E. D., Ferguson, J., Zeiss, C. R., Hatoum, N. (1994). Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. Journal of Allergy and Clinical Immunology 94(4):747-756.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Gross examinations and Lung histopathology
Duration:	Other (specify) 5 day initial exposure, challenge exposure after a 2-week rest
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	62970

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was definitively identified by name. A CASRN was not provided.
	Metric 2:	Test Substance Source	High	The test substance source was reported.
	Metric 3:	Test Substance Purity	Low	Purity was not defined and the test substance was not analytically verified by the performing laboratory.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	The study included most of the appropriate control groups, including air-exposed/PA-dust challenge, and air-exposed/air-challenge groups. However, a GPSA aerosol control was not included due to a technical challenge. This potentially impacts the ability to interpret the study results because some effects were only observed in animals challenged with PA-GPSA conjugate and not with the PA-dust alone, suggesting sensitization reactions could be due to GPSA alone. The study authors "recommend that this [challenge with GPSA alone] be included in future studies.
	Metric 5:	Positive Controls	N/A	Positive controls are generally included in respiratory sensitization studies. This study did not include a positive control; however, the test substance was a known respiratory sensitizer and this study was focused on determining a minimal effect level. No positive control was necessary.
	Metric 6:	Randomized Allocation of Animals	Low	Methods of allocating animals into the initial exposure groups was not described; however, it was noted that the animals sent to the second facility for testing were chosen at random (method not specified).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Details of atmosphere preparation were provided and were appropriate for the study type.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups. Animals were exposed, presumably whole body to PA dust in dynamic exposure chambers for the initial exposures. Airflow (305-375 L/min) was reported. The chambers size was specified. Challenge exposures were done on head-only, and animals were appropriately acclimated.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Target and analytical concentrations of PA-dust were reported along with MMAD and % respirable values. The concentration of the PA-dust challenge was also analytically verified. Some animals were challenged with a PA:GPSA conjugate. A molar ratio of 20:1 was reported, and a target concentration of 2.0 mg/m3 was provided; however, an analytical concentration of the conjugate was not provided.

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Study Citation:	Sarlo, K., Clark, E. D., Ferguson, J., Zeiss, C. R., Hatoum, N. (1994). Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. Journal of Allergy and Clinical Immunology 94(4):747-756.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Gross examinations and Lung histopathology
Duration:	Other (specify) 5 day initial exposure, challenge exposure after a 2-week rest
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	62970

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and durations were reported and appropriate for a respiratory sensitization study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The study authors justified the test concentrations. The goal of the study was to identify a minimal sensitizing levels or a no-effect level. However, in this study, immunogenic and allergenic responses were observed in animals from all dose groups, so a no-effect level was not determined. The authors concluded that the lowest concentration was at or near the minimal effect level, which is considerably lower than the recommended threshold limit value for exposure to PA (6.0 mg/m3).
	Metric 12:	Exposure Route and Method	Medium	Animals were exposed whole body for the initial exposures, and then were challenged with head-only exposures. Animals were appropriately acclimated for the head-only exposure scenarios. The authors did not explain why the initial exposures were also not performed as head-only exposures. The number of animals per cage during the whole-body exposures was not specified, but a dynamic chamber was used and the airflow (305-375 L/min) was reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test species was appropriate and had been used previously in similar studies. The species, strain, source, and starting body weights were reported. Animal age was not specified.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Food and water were provided ad libitum. No other animal husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals per group seemed appropriate for the outcomes of interest.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methodologies were adequately described and were appropriate and sensitive for the desired outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcomes were evaluated using the same protocol across groups. The information provided suggests that outcomes were consistently evaluated.
	Metric 18:	Sampling Adequacy	High	All animals were sampled for the outcomes of interest. The lungs of animals challenged with PA-GPSA conjugate were not histologically examined, but this is because no foci were observed during gross examinations.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not necessary for the outcomes of interest.
	Metric 20:	Negative Control Response	Medium	One or two lung foci were observed in 5/8 air-exposed and dust-challenged controls, although microscopic analysis showed no haemorrhage or inflammation. It is unclear why these animals showed foci, but it is not expected to have a significant impact on the study results.

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Study Citation:	Sarlo, K., Clark, E. D., Ferguson, J., Zeiss, C. R., Hatoum, N. (1994). Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. Journal of Allergy and Clinical Immunology 94(4):747-756.
Health Outcome(s):	Lung/Respiratory
Reported Health Effect(s):	Lung/Respiratory: Gross examinations and Lung histopathology
Duration:	Other (specify) 5 day initial exposure, challenge exposure after a 2-week rest
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	62970

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	A subset of animals was shipped to a separate facility for evaluation. The authors did not describe why this was necessary. However, the datasets were reported separately, and this is not expected to have a significant impact on the study results.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23: Data Presentation and Analysis	Low	For these outcomes (foci and lung histopathology) it is unclear that any statistical analysis was performed. The test only indicates that "a significant number of lung foci was defined as greater than 10." It is unclear how this cut-off was determined. However, sufficient data for foci in animals challenged with PA-dust are provided to allow for any outside statistical analysis. Histopathology data were inadequately described to allow for statistical analysis.
	Metric 24: Reporting of Data	Medium	The study included individual animal responses and reported the number of foci observed per animal. Histopathology data are shown as representative figures for the control and exposure group only. Additional results were described in the text.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Sarlo, K., Clark, E. D., Ferguson, J., Zeiss, C. R., Hatoum, N. (1994). Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. Journal of Allergy and Clinical Immunology 94(4):747-756.
Health Outcome(s):	Sensitization (Respiratory sensitization)
Reported Health Effect(s):	Sensitization (Respiratory sensitization): Respiratory sensitization
Duration:	Other (specify) 5 day initial exposure, challenge exposure after a 2-week rest
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	62970

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was definitively identified by name. A CASRN was not provided.
	Metric 2:	Test Substance Source	High	The test substance source was reported.
	Metric 3:	Test Substance Purity	Low	Purity was not defined and the test substance was not analytically verified by the performing laboratory.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	The study included most of the appropriate control groups, including air-exposed/PA-dust challenge, and air-exposed/air-challenge groups. However, a GPSA aerosol control was not included due to a technical challenge. This potentially impacts the ability to interpret the study results because some effects were only observed in animals challenged with PA-GPSA conjugate and not with the PA-dust alone, suggesting sensitization reactions could be due to GPSA alone. The study authors "recommend that this [challenge with GPSA alone] be included in future studies.
	Metric 5:	Positive Controls	N/A	Positive controls are generally included in respiratory sensitization studies. This study did not include a positive control; however, the test substance was a known respiratory sensitizer and this study was focused on determining a minimal effect level. No positive control was necessary.
	Metric 6:	Randomized Allocation of Animals	Low	Methods of allocating animals into the initial exposure groups was not described; however, it was noted that the animals sent to the second facility for testing were chosen at random (method not specified).
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Details of atmosphere preparation were provided and were appropriate for the study type.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups. Animals were exposed, presumably whole body to PA dust in dynamic exposure chambers for the initial exposures. Airflow (305-375 L/min) was reported. The chambers size was specified. Challenge exposures were done on head-only, and animals were appropriately acclimated.
	Metric 9:	Reporting of Doses/Concentrations	Medium	Target and analytical concentrations of PA-dust were reported along with MMAD and % respirable values. The concentration of the PA-dust challenge was also analytically verified. Some animals were challenged with a PA:GPSA conjugate. A molar ratio of 20:1 was reported, and a target concentration of 2.0 mg/m3 was provided; however, an analytical concentration of the conjugate was not provided.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and durations were reported and appropriate for a respiratory sensitization study.

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Study Citation:	Sarlo, K., Clark, E. D., Ferguson, J., Zeiss, C. R., Hatoum, N. (1994). Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. Journal of Allergy and Clinical Immunology 94(4):747-756.
Health Outcome(s):	Sensitization (Respiratory sensitization)
Reported Health Effect(s):	Sensitization (Respiratory sensitization): Respiratory sensitization
Duration:	Other (specify) 5 day initial exposure, challenge exposure after a 2-week rest
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	62970

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	The study authors justified the test concentrations. The goal of the study was to identify a minimal sensitizing levels or a no-effect level. However, in this study, immunogenic and allergenic responses were observed in animals from all dose groups, so a no-effect level was not determined. The authors concluded that the lowest concentration was at or near the minimal effect level, which is considerably lower than the recommended threshold limit value for exposure to PA (6.0 mg/m3).
	Metric 12:	Exposure Route and Method	Medium	Animals were exposed whole body for the initial exposures, and then were challenged with head-only exposures. Animals were appropriately acclimated for the head-only exposure scenarios. The authors did not explain why the initial exposures were also not performed as head-only exposures. The number of animals per cage during the whole-body exposures was not specified, but a dynamic chamber was used and the airflow (305-375 L/min) was reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test species was appropriate and had been used previously in similar studies. The species, strain, source, and starting body weights were reported. Animal age was not specified.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Food and water were provided ad libitum. No other animal husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals per group seemed appropriate for the outcomes of interest.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Outcome assessment methodologies were adequately described and were appropriate and sensitive for the desired outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	Outcomes were evaluated using the same protocol across groups. It was noted that sera was collected prior to the initial exposures to establish a baseline; however, a range was provided for later collections (18 to 24 hours). It is unclear which timepoints were used for which groups, and this could have a significant impact on the study results.
	Metric 18:	Sampling Adequacy	High	All animals were sampled for the outcomes of interest.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not necessary for the outcomes of interest.
	Metric 20:	Negative Control Response	Medium	There appeared to be a large variation across air-exposed control individuals that were challenged with PA-Dust (see Fig. 1). It is unclear whether this was expected, or if this type of variance for animal respiratory rates and % change in pressure is normal. The same measures in a separate group of air-exposed controls appeared to vary less (Fig. 2).
Domain 6: Confounding / Variable Control				

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Study Citation:	Sarlo, K., Clark, E. D., Ferguson, J., Zeiss, C. R., Hatoum, N. (1994). Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride. Journal of Allergy and Clinical Immunology 94(4):747-756.
Health Outcome(s):	Sensitization (Respiratory sensitization)
Reported Health Effect(s):	Sensitization (Respiratory sensitization): Respiratory sensitization
Duration:	Other (specify) 5 day initial exposure, challenge exposure after a 2-week rest
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	62970

Domain	Metric		Rating	Comments
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	A subset of animals was shipped to a separate facility for evaluation. The authors did not describe why this was necessary. However, the datasets were reported separately, and this is not expected to have a significant impact on the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All statistical methods were described and were appropriate for the datasets.
	Metric 24:	Reporting of Data	High	The study included individual animal responses for several endpoints. Other data were presented as means ± SE or incidences.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Vandebriel, R. J., Jong, De, W. H., Spiekstra, S. W., Dijk, Van, M., Fluitman, A., Garssen, J., Loveren, Van, H. (2000). Assessment of preferential T-helper 1 or T-helper 2 induction by low molecular weight compounds using the local lymph node assay in conjunction with RT-PCR and ELISA for interferon-gamma and interleukin-4. Toxicology and Applied Pharmacology 162(2):77-85.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA assay (auricular lymph node weight, cell number, proliferation [ex vivo], and cytokine release [ex vivo])
Duration:	Other (specify) Sensitization
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5160984

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by name as phthalic anhydride.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported (Sigma-Aldrich).
	Metric 3:	Test Substance Purity	High	The test substance purity (99.7%) was reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study included a concurrent negative control group that was exposed to a vehicle control.
	Metric 5:	Positive Controls	Medium	PA is a well-known respiratory irritant. The study also included TMA, a respiratory allergen that gave a positive response in a prior experiment.
	Metric 6:	Randomized Allocation of Animals	Low	No information on the methods of allocation of animals into test groups was reported. No other methods to control for modifying factors across groups were provided.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Details of test substance preparation were limited. The test substance was dissolved in 4:1 acetone: olive oil with a final concentration of 25% (w/v). Frequency and timing prior to application when the test substance was made up were not reported. Storage conditions were not reported. However, given that the study was short-term, this is unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study animals. The test substance was applied to the dorsum of both ears for three consecutive days.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal amount of test substance applied was reported. Animals were administered 25 µl of 25% PA onto each ear, daily for 3 days. Body weight was not reported, so a mg/kg dose cannot be estimated.
	Metric 10:	Exposure Frequency and Duration	High	Test animals were exposed via dermal application to the dorsum of both ears for three consecutive days.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	The study tested a single exposure group which was justified by the study authors. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	Animals were dermally exposed on the dorsal side of both ears for 3 consecutive days, which is appropriate for the test substance and for the study type.
Domain 4: Test Animals				

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Study Citation:	Vandebriel, R. J., Jong, De, W. H., Spiekstra, S. W., Dijk, Van, M., Fluitman, A., Garssen, J., Loveren, Van, H. (2000). Assessment of preferential T-helper 1 or T-helper 2 induction by low molecular weight compounds using the local lymph node assay in conjunction with RT-PCR and ELISA for interferon-gamma and interleukin-4. Toxicology and Applied Pharmacology 162(2):77-85.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA assay (auricular lymph node weight, cell number, proliferation [ex vivo], and cytokine release [ex vivo])
Duration:	Other (specify) Sensitization
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5160984

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	Medium	The study used male and female BALB/c mice. It is not clear which sex was used for these studies, or if both sexes were studied. The animals were sourced from either the study author's own breeding colony or Harlan/CPB. The age of the test animals was reported. Initial body weights were not reported; body weights could impact LLNA results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not reported. Study authors report that food and water were available ad libitum
	Metric 15:	Number of Animals per Group	Low	The number of animals exposed was not reported in the methods. Based on the sample sizes, there were likely 8 animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	A modified LLNA using ex vivo labeling of the proliferating LN cells was used, which deviates from the OECD 429 LLNA guidelines. However, the methodology was generally sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Details of the LLNA assessment protocol were reported and assessed consistently across groups.
	Metric 18:	Sampling Adequacy	High	The study reports data for 8 animals per group for the test substance group and 4 animals for the control (control group pooled 2 animals together due to low number of cells). OECD guidelines recommend a minimum of 4 animals per group for the LLNA assay.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was reported. SI and EC3 were not calculated.
	Metric 24:	Reporting of Data	Low	Data on lymph node weight and number of cells were not shown but reported to be significantly different in text. Proliferation and cytokine release data were shown graphically. SI was not calculated but could be estimated from presented data.

Additional Comments: None

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Study Citation:	Vandebriel, R. J., Jong, De, W. H., Spiekstra, S. W., Dijk, Van, M., Fluitman, A., Garssen, J., Loveren, Van, H. (2000). Assessment of preferential T-helper 1 or T-helper 2 induction by low molecular weight compounds using the local lymph node assay in conjunction with RT-PCR and ELISA for interferon-gamma and interleukin-4. Toxicology and Applied Pharmacology 162(2):77-85.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: LLNA assay (auricular lymph node weight, cell number, proliferation [ex vivo], and cytokine release [ex vivo])
Duration:	Other (specify) Sensitization
Chemical:	Phthalic anhydride- Parent compound
HERO ID:	5160984

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

Study Citation:	Larsen, S. T., Lund, R. M., Thygesen, P., Poulsen, O. M., Nielsen, G. D. (2003). Investigation of the adjuvant and immuno-suppressive effects of benzyl butyl phthalate, phthalic acid and benzyl alcohol in a murine injection model. Food and Chemical Toxicology 41(3):439-446.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: Levels of Ig in blood
Duration:	Short-term (>1-30 days) 19 days
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	673414

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	No concerns noted
	Metric 2:	Test Substance Source	High	No concerns noted regarding source of test substance
	Metric 3:	Test Substance Purity	High	No concerns noted regarding purity of test substance
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	This study did not have a control group that was not treated with ovalbumin/primary adjuvant; however, the study was designed to evaluate the adjuvant effect of phthalic acid against ovalbumin only, so I rated this metric as low, rather than unacceptable.
	Metric 5:	Positive Controls	N/A	not relevant for this outcome
	Metric 6:	Randomized Allocation of Animals	Medium	The study reported that animals were randomly allocated into study groups (including the control group)
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Test substance preparation and storage conditions were insufficiently documented.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups
	Metric 9:	Reporting of Doses/Concentrations	High	Reports doses "PA was administered in the concentration 1,064 mg/ml, which corresponds 6.4 mM"
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Only one dose level of phthalic acid was tested.
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were suited to the test substance (see above) for the purposes of this experiment, which required i.p. injection
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The test animal species, strain, sex, age, and starting body weight were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure
	Metric 15:	Number of Animals per Group	Low	The number of animals per study group was not reported

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Study Citation:	Larsen, S. T., Lund, R. M., Thygesen, P., Poulsen, O. M., Nielsen, G. D. (2003). Investigation of the adjuvant and immuno-suppressive effects of benzyl butyl phthalate, phthalic acid and benzyl alcohol in a murine injection model. Food and Chemical Toxicology 41(3):439-446.
Health Outcome(s):	Immune/Hematological
Reported Health Effect(s):	Immune/Hematological: Levels of Ig in blood
Duration:	Short-term (>1-30 days) 19 days
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	673414

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	Other measures of immune response beyond Ig levels in blood were not evaluated.
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol (e.g., administration of the boosters) for outcome assessment were not reported clearly.
	Metric 18:	Sampling Adequacy	Low	Sampling was not adequately described for the groups treated or not with phthalic acid.
	Metric 19:	Blinding of Assessors	N/A	outcomes are not subjective and blinding of assessors is not necessary
	Metric 20:	Negative Control Response	N/A	Use for assays where no control is required
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods (including any calculations or data transformations) were clearly described or had only minor omissions and were appropriate for the datasets
	Metric 24:	Reporting of Data	Low	The number of animals evaluated for effects was not reported.

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Larsen, S. T., Lund, R. M., Thygesen, P., Poulsen, O. M., Nielsen, G. D. (2003). Investigation of the adjuvant and immuno-suppressive effects of benzyl butyl phthalate, phthalic acid and benzyl alcohol in a murine injection model. Food and Chemical Toxicology 41(3):439-446.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Short-term (>1-30 days) 19 days
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	673414

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	No concerns noted
	Metric 2:	Test Substance Source	High	No concerns noted regarding source of test substance
	Metric 3:	Test Substance Purity	High	No concerns noted regarding purity of test substance
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	This study did not have a control group that was not treated with ovalbumin/primary adjuvant; however, the study was designed to evaluate the adjuvant effect of phthalic acid against ovalbumin only, so I rated this metric as low, rather than unacceptable.
	Metric 5:	Positive Controls	N/A	not relevant for this outcome
	Metric 6:	Randomized Allocation of Animals	Medium	The study reported that animals were randomly allocated into study groups (including the control group)
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Test substance preparation and storage conditions were insufficiently documented.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups
	Metric 9:	Reporting of Doses/Concentrations	High	Reports doses "PA was administered in the concentration 1064 mg/ml, which corresponds 6.4 mM"
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Only one dose level of phthalic acid was tested.
	Metric 12:	Exposure Route and Method	Medium	The route and method of exposure were reported and were suited to the test substance (see above) for the purposes of this experiment, which required i.p. injection.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The test animal species, strain, sex, age, and starting body weight were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle, diet, water availability) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number of Animals per Group	Low	The number of animals per study group was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	Other measures of nutritional effects were not evaluated.

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Study Citation:	Larsen, S. T., Lund, R. M., Thygesen, P., Poulsen, O. M., Nielsen, G. D. (2003). Investigation of the adjuvant and immuno-suppressive effects of benzyl butyl phthalate, phthalic acid and benzyl alcohol in a murine injection model. Food and Chemical Toxicology 41(3):439-446.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight
Duration:	Short-term (>1-30 days) 19 days
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	673414

Domain	Metric		Rating	Comments
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol (e.g., administration of the boosters) for outcome assessment were not reported clearly.
	Metric 18:	Sampling Adequacy	Low	Sampling was not adequately described for the groups treated or not with phthalic acid.
	Metric 19:	Blinding of Assessors	N/A	outcomes are not subjective and blinding of assessors is not necessary
	Metric 20:	Negative Control Response	N/A	no control is required for this assay
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences (or identified only minor differences) among study groups in the above listed confounding factors.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods (including any calculations or data transformations) were clearly described or had only minor omissions and were appropriate for the dataset(s)
	Metric 24:	Reporting of Data	Low	The number of animals evaluated for effects was not reported and quantitative data is not presented for body weights (negative results are reported in the text)

Additional Comments: None

Overall Quality Determination — Medium

Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Subcutaneous
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	65818

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was reported as phthalic acid. A CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source was Fisher Scientific, Fair Lawn, NJ). Batch/lot number was not identified.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Appropriate negative controls were used for most of the tests included. A vehicle-only control group was included for the injection regimen. A GPSA alone (negative control) group was used for ACA, PCA, and ELISA tests. However, the intratracheal challenge was done with PA-GPSA only; a GPSA alone group was not included, but would have been appropriate.
	Metric 5:	Positive Controls	Medium	Phthalic anhydride was included which is a known sensitizer and gave positive responses.
	Metric 6:	Randomized Allocation of Animals	Low	The study does not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The storage conditions of test chemical were not reported. Test substance was dissolved in olive oil prior to injections; no details are provided. The lack of details in unlikely to substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	The test substance was delivered subcutaneously 2 times/week for 4 weeks. The volume administered was consistent. The anatomical location where the test substance was delivered was not reported.
	Metric 9:	Reporting of Doses/Concentrations	Low	Doses were reported as a molar dose (400 uL 6.7 x10-4 M) for ease of comparison with other chemicals tested. The volume administered is reported. Initial body weights were reported, but it is unclear how much weight the animals gained throughout the 28 days; therefore, calculated doses in mg/kg-day based on initial body weights would likely not represent the actual doses. 100uL of the PA-GPSA conjugate (500 ug/mL) was delivered intratracheally.
	Metric 10:	Exposure Frequency and Duration	High	The frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	Only one dose group was studied, and negative results were obtained. It is unclear if a higher dose may have elicited a response. Dosing injections were chosen based on initial range-finding studies using weight/volume doses of phthalic anhydride. Phthalic acid was used as a control group in a study focused on phthalic anhydride.

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Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Subcutaneous
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	65818

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Medium	The exposure route and method were appropriate for the test chemical and aim of the study. Injection site on the animals was not reported.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	The test animal source, sex, species, strain and starting body weights were reported. Age or life stage was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Food and water were available ad libitum. No other husbandry conditions were reported.
	Metric 15:	Number of Animals per Group	Medium	The number of animals/group (10/group) was appropriate. Control group included 30 animals (a combination of controls from tests done on 3 different chemicals)
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Low	Phthalic acid would not form conjugates and, therefore, was negative in Tier 2 testing; therefore, all of the tests to assess immunogenicity were done using PA-GPSA (or GPSA as a control). Because challenges weren't done with phthalic acid, this study cannot assess the sensitisation potential for phthalic acid exposures. Instead, this study only assesses whether phthalic acid can sensitize animals to PA-GPSA exposure. Details on the PCA test were confusing and not clearly outlined.
	Metric 17:	Consistency of Outcome Assessment	High	Based on the information available, outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate and all animals were accounted for in data tables.
	Metric 19:	Blinding of Assessors	Low	Blinding was not reported. Changes in respiratory rate was visually monitored for 10 minutes. The assessor was looking for the number of diaphragmatic contractions. This could be subjective. Other outcomes were not subjective in nature.
	Metric 20:	Negative Control Response	Low	The study did not report ACA test or PCA test, or ELISA results for the GPSA alone controls.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Not all information was reported to determine confounding, reported information did not identify any differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods were reported and appropriate; or responses were reported as positive/negative.
	Metric 24:	Reporting of Data	Medium	Data were reported fully for all outcomes of interest except for the GPSA controls (see Metric 20). Incidence data and mean +/- variance were reported.

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Study Citation:	Sarlo, K., Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Toxicological Sciences 18(1):107-114.
Health Outcome(s):	Sensitization
Reported Health Effect(s):	Sensitization: Respiratory response, serum IgG and IgG1a
Duration:	Other (specify) Subcutaneous
Chemical:	Phthalic anhydride- Metabolite: Phthalic acid
HERO ID:	65818

Domain	Metric	Rating	Comments

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Lake, B., Gangolli, S., Grasso, P., Lloyd, A. (1975). Studies on the hepatic effects of orally administered di-(2-ethylhexyl) phthalate in the rat. Toxicology and Applied Pharmacology 32(2):355-367.
Health Outcome(s):	Hepatic/Liver
Reported Health Effect(s):	Hepatic/Liver: No significant changes in liver weight or biochemistry were observed, and no histochemical or ultrastructural changes were observed.
Duration:	Short-term (>1-30 days) 7 days
Chemical:	Phthalic anhydride- Metabolite: 1,2-phthalic acid
HERO ID:	699519

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	1,2-Phthalic acid (phthalic acid) was identified as the test substance. No other details were provided regarding the characterization of the test substance (CASRN, structure, form).
	Metric 2:	Test Substance Source	High	The test substance was obtained from British Drug Houses in Poole, Dorset, England.
	Metric 3:	Test Substance Purity	Medium	The purity was reported as laboratory reagent grade. Impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	Negative controls were administered 0.5 mL/100g bw of the vehicle (corn oil). It was not specified if corn oil was also used as the vehicle for phthalic acid.
	Metric 5:	Positive Controls	N/A	Positive control not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Information on preparation and storage was not reported, but the omission of details are unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration are incompletely reported, but the missing information is unlikely to have a substantial impact on results. Volume administered was not specified.
	Metric 9:	Reporting of Doses/Concentrations	Medium	One oral dose of 850 mg/kg bw/day was administered to rats. It was not reported if this was a nominal or measured dose.
	Metric 10:	Exposure Frequency and Duration	High	Seven daily oral doses of the test substance were administered.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	Only one exposure group was used, and the justification for this dose selection was not provided and no significant effects were observed.
	Metric 12:	Exposure Route and Method	Medium	The route of exposure was appropriate for the outcome (oral exposure to examine liver effects). The method of oral exposure was not specified for phthalic acid; doses of DEHP were administered via oral gavage in the same study.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Young (age not specified) male Wistar albino rats were used in this study. Bodyweights were between 100-120 g. The source of the animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Animals were housed 6/cage at 20 deg. C with 50% humidity and were given free access to food and water. Light-dark cycles were not described.

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Study Citation:	Lake, B., Gangolli, S., Grasso, P., Lloyd, A. (1975). Studies on the hepatic effects of orally administered di-(2-ethylhexyl) phthalate in the rat. Toxicology and Applied Pharmacology 32(2):355-367.
Health Outcome(s):	Hepatic/Liver
Reported Health Effect(s):	Hepatic/Liver: No significant changes in liver weight or biochemistry were observed, and no histochemical or ultrastructural changes were observed.
Duration:	Short-term (>1-30 days) 7 days
Chemical:	Phthalic anhydride- Metabolite: 1,2-phthalic acid
HERO ID:	699519

Domain	Metric		Rating	Comments
	Metric 15:	Number of Animals per Group	Low	6 animals were used in each group based on the results in Table 2. No additional details were provided.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	Liver weights, biochemistry, and liver histopathology were examined to evaluate effects on the liver. Methods were described. AST, ALT, and ALP were not evaluated.
	Metric 17:	Consistency of Outcome Assessment	Low	Timing of each outcome assessment and details of the study protocol execution were not provided.
	Metric 18:	Sampling Adequacy	High	6 animals per group were evaluated for each outcome (Table 2).
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for any of the liver endpoints evaluated.
	Metric 20:	Negative Control Response	High	Data for the control group was provided in Table 2 and appeared appropriate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	Bodyweight changes and food/water consumption were not evaluated.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure.
	Metric 23:	Data Presentation and Analysis	Low	Student's t-test was used as the statistical model, but no other information was provided. Data that was provided was not sufficient to conduct an independent statistical analysis.
	Metric 24:	Reporting of Data	High	Data for exposure-related findings were presented for all outcomes by exposure group (mean +/- SE).

Additional Comments: None

Overall Quality Determination — Medium