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Draft Human Health Hazard Assessment for Phthalic Anhydride

Technical Support Document for the Draft Risk Evaluation

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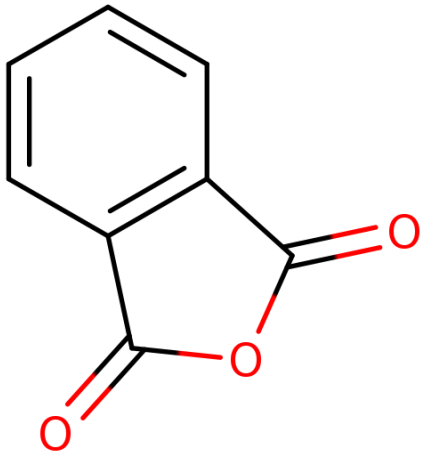

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KEY ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists (U.S.)
ADME	Absorption, distribution, metabolism and excretion
AIHA	American Industrial Hygiene Association
ALP	Alkaline phosphatase
AOP	Adverse outcome pathway
ARE	Antioxidant response element
AUC	Area under the concentration-time curve
BALB	Albino, laboratory-bred strain mice
BBP	Butyl benzyl phthalate
CASRN	Chemical abstracts service registry number
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
C_{max}	Peak serum concentrations
DBP	Dibutyl phthalate
DECOS	Dutch Expert Committee on Occupational Standards
DEHP	Diethylhexyl phthalate
DER	Data evaluation record
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
DNA	Deoxyribonucleic acid
DPRA	Direct peptide reactivity assay
DV	Decision value
EC_3	Estimated concentration needed to produce a stimulation index of 3
ELISA	Enzyme-linked immunosorbent assay

EPA	Environmental Protection Agency (U.S.)
ERK	ERK active, functional form (for protein assay)
EU	European Union
F344	Fischer 344 rat
FRN	Federal register notice
GD	Gestation day
GHS	Globally Harmonised System of Classification and Labelling of Chemicals
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GOT	Glutamic oxaloacetic transaminase
GPMT	Guinea Pig Maximization Test
GSD	Geometric standard deviation
h-CLAT	Human cell line activation test
HEC	Human equivalent concentration
HED	Human equivalent dose
HPPT	Human predictive patch test
HSA	Human serum albumin
IATA	Integrated Approaches to Testing and Assessment
ICE	Integrated Chemical Environment
Ig	Immunoglobulin
IL	Interleukin
i.p.	Intraperitoneal
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IRIS	Integrated Risk Information System (U.S.)
kDPRA	Kinetic DPRA
KE	Key event
LDH	Lactate dehydrogenase
LLNA	Local lymph node assay
LOAEL	Lowest-observed-adverse-effect level
LOAEC	Lowest-observed-adverse-effectconcentration
MA	Maleic anhydride
MHC	Major histocompatibility complex
MIE	Molecular initiating event
MMAD	Mass median aerodynamic diameter
MOA	Mode of action
MOE	Margin of exposure
MPPD	Multi-Path Particle Dosimetry
MTD	Maximum tolerated dose
NAM	New Approach Methodology
NCI	National Cancer Institute
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NICNAS	National Industrial Chemicals Notification and Assessment Scheme (Australia)
NIOSH	National Institute for Occupational Safety and Health (U.S.)
NOAEL	No-observed-adverse-effect level
NOAEC	No-observed-adverse-effectconcentration
NTP	National Toxicology Program
OCSPP	Office of Chemical Safety and Pollution Prevention (EPA)
OECD	Organisation for Economic Co-operation and Development
OEHHA	Office of Environmental Health Hazard Assessment

OEL	Occupational exposure limit
OPP	Office of Pesticide Programs (EPA)
OPPT	Office of Pollution Prevention and Toxics (EPA)
OSHA	Occupational Safety and Health Administration (U.S.)
PBPK	Physiologically based pharmacokinetic
PBS	Phosphate-buffered saline
PECO	Population, exposure, comparator, and outcome
PEL	Permissible exposure limit
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal day
POD	Point of departure
PPRTV	Provisional Peer-Reviewed Toxicity Value
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals)
REL	Recommended exposure limit
SACC	Science Advisory Committee on Chemicals
SARA-ICE	Skin Allergy Risk Assessment – Integrated Chemical Environment
SCE	Sister chromatid exchange
SCOEL	Scientific Committee on Occupational Exposure Limits
SD	Sprague-Dawley (rats)
SEM	Standard error of measurement
SI	Stimulation index
SIC	Specific inhalation challenge
SISAS	Societa Italiana Serie Acetia Sintetica
SPUR	SARA-ICE prediction uncertainty ratio
STEL	Short-term exposure limit
SVM	Support Vector Machine
TCR	T-cell receptor
TG	Test Guideline
Th1	Type 1
Th2	Type 2
TLV	Threshold limit value
TMA	Trimellitic anhydride
TOMA	Tabershaw Occupational Medicine Associates
TSCA	Toxic Substances Control Act
TSD	Technical support document
TSLP	Thymic stromal lymphopoietin
TSLPR	Thymic stromal lymphopoietin receptor
TWA	Time-weighted average
UF	Uncertainty factor
UF_A	Interspecies uncertainty factor
UF_H	Intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL (or LOAEC-to-NOAEC) uncertainty factor
U.S.	United States
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

SUMMARY

This technical support document (TSD) is part of the *Draft Risk Evaluation for Phthalic Anhydride* (U.S. EPA, 2026i) (also called the "draft risk evaluation"; see also public docket, EPA-HQ-OPPT-2018-0459). This draft TSD describes the use of reasonably available information to identify the non-cancer, genotoxicity, and cancer human health hazards associated with exposure to phthalic anhydride and *ortho*-phthalic acid (or *o*-phthalic acid). This document also describes the points of departure (PODs) used to estimate risks from relevant exposures in the draft risk evaluation. For workers and consumers, the U.S. Environmental Protection Agency (EPA or the Agency) is quantitatively assessing dermal and inhalation (but not oral) exposures to phthalic anhydride. For the general population screening-level assessment, EPA is evaluating oral and inhalation exposures from Toxic Substances Control Act (TSCA) releases of phthalic anhydride. Given that phthalic anhydride rapidly hydrolyzes to *o*-phthalic acid in the presence of moisture (*i.e.,* hydrolysis half-life of 30–90 seconds, with complete hydrolysis after ≈8 minutes), the general population is expected to be exposed to *o*-phthalic acid, not phthalic anhydride, from TSCA releases.

TSCA section 4(h)(1) promotes the use of alternative test methods that reduce or replace vertebrate animals to the extent practicable when scientifically justified and consistent with TSCA's science standards. Consistent with this mandate, EPA, on January 22, 2026, recommitted to phasing-out mammalian animal testing and further incorporating New Approach Methods (NAMs) into chemical risk evaluations.[1] This assessment exemplifies this commitment by applying several computational approaches, *in vitro* studies in human tissues, and adverse outcome pathways (AOPs) throughout the hazard assessment. The data supporting this draft risk evaluation is significantly informed by EPA's collaborations with organizations such as the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), animal welfare organizations, and *in vitro* method developers (*e.g.,* Invitrolize) who enabled the inclusion of cutting edge science, consistent with EPA's statutory mandate to use the best available science.

EPA identified dermal (Section 4.2) and respiratory sensitization (Section 4.3) as the most sensitive health effects following dermal and inhalation exposure to phthalic anhydride, respectively. The Agency has robust confidence that these are the most sensitive health effects. In contrast, *o*-phthalic acid is not a dermal or respiratory sensitizer. These findings are based on observational studies in exposed worker populations and experimental studies in laboratory animals and with NAMs. Notably, previous assessments, including by the Organization for Economic Co-operation and Development (OECD, 2005), Australia National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2013), Health Canada (2019), and the American Conference of Government Industrial Hygienists (ACGIH, 2025) have also concluded that phthalic anhydride is a dermal and respiratory sensitizer. Furthermore, phthalic anhydride is classified (Globally Harmonised System [GHS]) as Skin Sens. 1 (H317: May cause an allergic skin reaction) and Resp. Sens. 1 (H344: May cause allergy or asthma symptoms or breathing difficulties if inhaled) in the European Union (EU).[2]

For dermal exposures to phthalic anhydride, EPA identified dermal sensitization as the most sensitive human health hazard (Section 4.2). This conclusion is supported by reasonably available evidence from human studies of exposed workers and consumers, animal toxicology studies (*i.e.,* local lymph node

[1] U.S. Environmental Protection Agency. (2026, January 22). Administrator Zeldin Gets EPA Back on Track to Eliminate Animal Testing After Biden Admin Halted Phase Out [Press Release], accessed March 25, 2026. https://www.epa.gov/newsreleases/administrator-zeldin-gets-epa-back-track-eliminate-animal-testing-after-biden-admin

[2] https://chem.echa.europa.eu/100.001.461/overview?searchText=85-44-9, accessed February 23, 2026.

assays [LLNAs], Guinea Pig Maximization Tests [GPMTs], Buehler tests), as well as mechanistic *in vitro* assays (*i.e.,* U-SENS and GARDskin assays) and *in chemico* assays (*i.e.,* Direct Peptide Reactivity Assay [DPRA] and kinetic DPRA [kDPRA]). Available data were evaluated in the context of the OECD (2014) adverse outcome pathway (AOP) for skin sensitization (Section 4.2.1.1), which describes the series of linked key events (KEs) at different levels of biological organization (*e.g.,* cell, tissue, organ) that ultimately manifest as the adverse outcome of skin sensitization. In the OECD's AOP, the linked KEs occur during the immune priming phase following initial exposure (*i.e.,* induction), while the adverse outcome occurs after subsequent exposure to reflect that skin sensitization is a biphasic response (*i.e.,* an induction and subsequent elicitation phase).

Consistent with OECD's *Guideline No. 497: Defined approaches on skin sensitization* (OECD, 2025b), EPA used the Skin Allergy Risk Assessment – Integrated Chemical Environment (SARA-ICE) model to derive a dermal POD based on skin sensitization expected in humans. SARA-ICE is a computational, NAMs-based approach that provides a non-animal, human-derived option for hazard and risk assessment. Using SARA-ICE, EPA derived a draft POD of 45 μg/cm^2 (Section 4.2.1.3). The draft POD is based on the SARA-ICE predicted estimate of the ED01, which is the effective dose at which there is a 1% chance of inducing sensitization in a human predictive patch test (HPPT). A total uncertainty factor (UF) of 1 was selected for use as the benchmark margin of exposure (MOE) (based on a intraspecies UF [UF_H] of 1, because POD is based on the SARA-ICE model ED01 estimate, which captures the variability in the human population for phthalic anhydride).

For the inhalation route, EPA concluded that respiratory sensitization was the most sensitive human health hazard (Section 4.3). This conclusion is based on the best available science and weight of scientific evidence across reasonably available data from epidemiology studies of exposed workers, experimental studies of laboratory animals, and *in vitro* mechanistic data. Similar to the dermal section of this draft TSD, the inhalation section is organized in the context of an AOP for respiratory sensitization, AOP 39 (Section 4.3.1.1). AOP 39 describes the series of linked KEs at different levels of biological organization in the respiratory tract (*e.g.,* cell, tissue, organ) that ultimately manifest as the adverse outcome of an allergic respiratory hypersensitivity response. In that AOP, the linked KEs occur during the immune priming phase following initial exposure (*i.e.,* induction), while the adverse outcome occurs after subsequent exposure to reflect the biphasic response of respiratory sensitization (*i.e.,* an induction and subsequent elicitation phase). Unlike dermal sensitization, there are not yet validated approaches or *in vivo* or *in vitro* test methods for identifying respiratory sensitizers for use in a regulatory context. Although AOP 39 sensitization is used as an organizing principle in this 2026 draft human health hazard assessment for phthalic anhydride, it remains under development and is not yet endorsed by OECD.

EPA also considered analog data in support of the conclusion that phthalic anhydride is a respiratory sensitizer. Trimellitic anhydride (TMA) was identified as an analog due to its structural similarity, comparable physical, chemical, and environmental fate properties, and available data indicating that both TMA and phthalic anhydride are toxicologically similar as dermal and respiratory sensitizers (Appendix B).

EPA integrated available dose-response information from candidate PODs for phthalic anhydride with existing hazard values from other organizations, including other regulatory agencies (Section 4.3.1.3. The Agency is proposing a draft POD for phthalic anhydride of 0.4 mg/m^3 from an occupational exposure study by Nielsen et al. (1988). The POD is based on a lowest-observed-adverse-effect concentration (LOAEC) for increased incidence of respiratory symptoms and increased serum-specific IgG consistent with respiratory sensitization. A total uncertainty factor (UF) of 30 times (30×) was

selected for use as the benchmark MOE (based on an UF_H of 10× to account for human variability, and a LOAEC-to-NOAEC (no-observed-adverse-effect concentration) UF (UF_L) of 3× to account for the lack of a NOAEC in the critical study).

The general population is expected to be primarily exposed to *o*-phthalic acid from TSCA releases of phthalic anhydride. This is because phthalic anhydride rapidly hydrolyzes to *o*-phthalic acid in the presence of moisture. *o*-Phthalic acid is not a dermal or respiratory sensitizer and no hazards were identified for the dermal and inhalation exposure routes. EPA evaluated hazards associated with oral exposure to *o*-phthalic acid in addition to phthalic anhydride (Section 4.1). Reasonably available studies of *o*-phthalic acid were primarily limited to the oral exposure route. Of the 11 reasonably available oral studies (9 in rats; 2 in mice), 5 reported no-effect levels exceeding 1,000 mg/kg-day following gavage or dietary exposure and lowest-observed-adverse-effect levels (LOAELs) of 4 studies reflecting decreases in body weight or food consumption ranging from 250 to 5,000 mg/kg-day.

Overall, EPA has preliminarily concluded that phthalic anhydride and *o*-phthalic acid have low systemic toxicity via the oral route of exposure (Section 4.1.3). Notably, OECD (2005), Australia NICNAS (2013), and Health Canada (2019) have also concluded that phthalic anhydride has low systemic toxicity via the oral route of exposure. For exclusive use in the general population screening-level assessment, EPA is proposing an oral POD derived from a NOAEL of 278 mg/kg-day (human equivalent dose [HED] = 66 mg/kg-day) based on decreased body weight gain in male F344 rats fed diets containing phthalic anhydride for 2 years (NCI, 1979). Although the key study used to derive the proposed oral POD is based on dietary exposure to phthalic anhydride, this POD is considered suitable for use in characterizing risk in its general population screening-level risk assessment because phthalic anhydride and *o*-phthalic acid are toxicologically similar via the oral exposure route. That is, both chemicals exhibit low systemic toxicity and cause similar effects, which are primarily limited to decreases in body weight gain, terminal body weight, and food consumption. This is in contrast to the inhalation and dermal exposure routes, where phthalic anhydride and *o*-phthalic acid exhibit different hazard profiles (*i.e.,* phthalic anhydride is a dermal and respiratory sensitizer; *o*-phthalic acid is not). A total UF of 30× was selected for use as the benchmark MOE (UF_H = 10×; interspecies UF [UF_A] = 3×).

For use in the general population screening assessment, no data are reasonably available for the inhalation route for *o*-phthalic acid that are suitable for deriving route-specific PODs, therefore, EPA is using the oral POD (derived for phthalic anhydride) to estimate risks from inhalation exposures to *o*-phthalic acid by extrapolating the oral HED to inhalation human equivalent concentrations (HECs) assuming the human body weight and breathing rates of an individual at rest (U.S. EPA, 1994). The HEC for use in the general population screening assessment is 358 mg/m^3 (52.6 ppm), and a total UF of 30× was selected for use as the benchmark MOE (UF_H = 10×; [UF_A] = 3×) (see Section 4.1.5). According to EPA guidelines, route-to-route extrapolation is appropriate in the absence of a suitable physiologically based pharmacokinetic (PBPK) model and/or absence of data to determine dosimetry via inhalation in cases where there is not portal of entry toxicity (U.S. EPA, 1994).

EPA did not identify potential for genotoxicity (Section 5) or carcinogenicity (Section 6) for phthalic anhydride and/or *o*-phthalic acid and preliminarily concluded that phthalic anhydride is *Not Likely to Be Carcinogenic to Humans* for the oral exposure route. Consistent with this cancer classification, EPA is not conducting a dose-response assessment for phthalic anhydride or *o*-phthalic acid or evaluating phthalic anhydride for carcinogenic risk to humans under TSCA.

EPA is soliciting comments from the independent Science Advisory Committee on Chemicals (SACC) and the public on the cancer and non-cancer hazard identification, dose-response, and weight of

evidence analyses, as well as the proposed PODs for use in risk characterization in the *Draft Risk Evaluation for Phthalic Anhydride* (U.S. EPA, 2026i). In particular, EPA is seeking SACC and public input on the dose-response assessment used to derive the draft dermal POD based on skin sensitization—including use of the OECD skin sensitization AOP as organizing framework for integration of *in chemico*, *in vitro*, and *in vivo* data, as well as EPA's application of the SARA-ICE dose-response model. The Agency is also seeking input on its use of AOP as an organizational framework for human evidence and evidence from *in chemico*, *in vitro*, and *in vivo* test systems for respiratory sensitization, as well as its dose-response assessment to derive the draft inhalation POD for phthalic anhydride based on respiratory sensitization.

1 INTRODUCTION

This draft TSD presents the draft human health hazard assessment in support of the TSCA *Draft Risk Evaluation for Phthalic Anhydride* (U.S. EPA, 2026i) conducted under the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amended TSCA on June 22, 2016. The law includes statutory requirements and deadlines for actions related to conducting risk evaluations of existing chemicals. In December 2019, EPA designated phthalic anhydride as a high-priority substance for risk evaluation following the prioritization process as required by Section 6(b) of TSCA and implementing regulations (40 CFR 702) (U.S. EPA, 2019). The Agency published the final scope document for phthalic anhydride in August 2020 (U.S. EPA, 2020a). Following publication of the final scope, one of the next steps in the TSCA risk evaluation process is to identify and characterize the human health hazards of phthalic anhydride and conduct a dose-response assessment to determine the toxicity values to be used to estimate risks from phthalic anhydride exposures.

As discussed in the final scope document (U.S. EPA, 2020a) and in the *Draft Physical Chemistry and Fate and Transport Assessment for Phthalic Anhydride* (U.S. EPA, 2026h), phthalic anhydride rapidly hydrolyzes to 1,2,-benzenedicarboxylic acid, also known as *ortho*-phthalic acid or *o*-phthalic acid (CASRN 88-99-3), when allowed contact with water or moisture. This transformation is immediate and the hydrolysis half-life is estimated to be between 30 to 90 seconds, depending upon pH, and complete hydrolysis is achieved in approximately 8 minutes in simulated seawater conditions or physiological fluids (U.S. EPA, 2026h). Given the rapid hydrolysis of phthalic anhydride to *o*-phthalic acid, EPA considered human health hazard data for both phthalic anhydride and *o*-phthalic acid in this draft human health hazard assessment.

This draft assessment/TSD for phthalic anhydride summarizes the non-cancer (Section 4) and cancer (Section 6) hazards associated with exposure to phthalic anhydride and *o*-phthalic acid and proposes non-cancer toxicity values to be used to estimate risks from phthalic anhydride exposures. Section 2 presents EPA's approach and methodology for the human health hazard assessment. The toxicokinetics of phthalic anhydride are discussed in Section 3. The hazard identification and evidence integration for the oral, dermal, and inhalation routes of exposure are presented in Sections 4.1, 4.2, and 4.3, respectively. Sections 5 and 6 summarize available genotoxicity and cancer hazard data. An analysis of potentially exposed or susceptible subpopulations (PESS) along with considerations for aggregate exposure are described in Section 7. Finally, the draft human hazard values to be used for risk estimates are summarized in Section 8. There are also five appendices.

2 APPROACH AND METHODOLOGY

EPA utilized systematic review processes to search, screen, evaluate, extract, and integrate reasonably available information to make conclusions about relevant adverse health effects from both phthalic anhydride and *o*-phthalic acid exposure. Following evidence integration, EPA performed dose-response analysis to derive hazard values for use in risk characterization. The Agency then evaluated the weight of scientific evidence for each aspect of the assessment and determined overall confidence ratings for each critical hazard outcome. The generalized process for conducting human health assessments under TSCA is presented below in Figure 2-1.

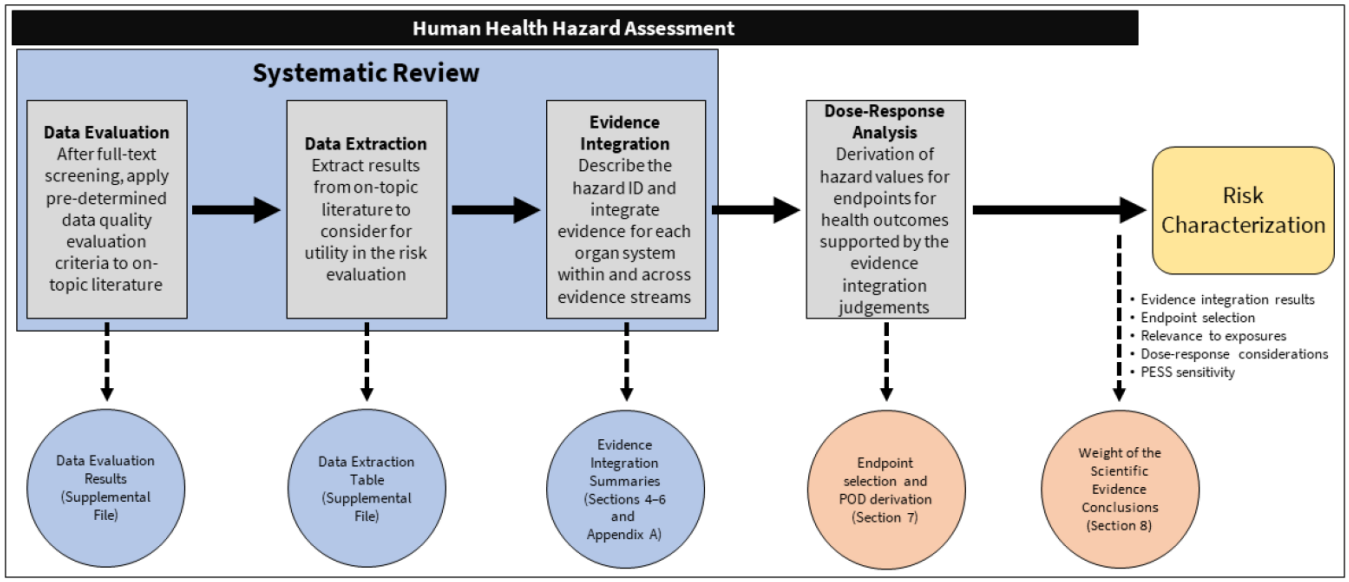

Figure 2-1. EPA Approach to Hazard Identification, Evidence Integration, and Dose-Response Analysis for Phthalic Anhydride

2.1 Systematic Review

The searching and screening steps of the systematic review process for phthalic anhydride generally followed the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances, Version 1.0: A Generic TSCA Systematic Review Protocol with Chemical-Specific Methodologies* (also called the "Draft Systematic Review Protocol") (U.S. EPA, 2021) covering all reasonably available literature published through October 2025. Full details and screening results for all the identified studies, as well as subsequent identification and review of key studies (*i.e.,* population, exposure, comparator, and outcome [PECO] relevant), are described in the *Draft Systematic Review Protocol for Phthalic Anhydride* (U.S. EPA, 2026j), which also presents EPA's process for considering and incorporating new phthalic anhydride and *o*-phthalic acid literature.

As described in the *Draft Systematic Review Protocol for Phthalic Anhydride* (U.S. EPA, 2026j), all studies that were used quantitatively by EPA for the current assessment, as well as studies used quantitatively in previous assessments, underwent data evaluation and extraction as part of the systematic review process. For studies used quantitatively in the dermal sensitization dose-response assessment in Section 4.2.1.3, EPA developed data evaluation records (DERs) as described in the *Draft Systematic Review Protocol* (U.S. EPA, 2026j). DERs were developed for *in chemico* direct peptide reactivity assays (DPRAs) and kinetic DPRAs (kDPRA), as well as for *in vitro* human cell line activation test (h-CLAT) and *in vitro* KeratinoSens assays and local lymph node assays (LLNAs). DERs were developed for these study types because EPA's data quality evaluation process is not intended to

evaluate these types of *in vivo*, *in vitro*, and *in chemico* assays. For studies that went through data evaluation and extraction, formal data evaluation results can be found in *Draft Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Phthalic Anhydride* (U.S. EPA, 2026d) and *Draft Data Quality Evaluation Information for Human Health Hazard Epidemiology for Phthalic Anhydride* (U.S. EPA, 2026e). Formal extraction results can be found in *Draft Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Phthalic Anhydride* (U.S. EPA, 2026c). For studies in which DERs were developed, DERs can be found in *Draft Data Evaluation Record Information for in chemico, in vitro, and in vivo Assays for Human Health Hazard for Phthalic Anhydride* (U.S. EPA, 2026b). Appendix C of the draft risk evaluation lists all TSDs and supplemental files included in the risk evaluation package.

Epidemiologic Data Considerations

As discussed in the *Draft Systematic Review Protocol for Phthalic Anhydride* (U.S. EPA, 2026j), EPA identified several epidemiology studies evaluating associations between occupational exposure to phthalic anhydride and various health outcomes, as well as epidemiology studies evaluating associations between urinary, serum, plasma, or semen *o*-phthalic acid levels and various health outcomes. *o*-Phthalic acid is a common metabolite of phthalic anhydride and many phthalate diesters that humans are regularly exposed to, including (but not limited to) diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), diisobutyl phthalate (DIBP), diisononyl phthalate (DINP), and diisodecyl phthalate (DIDP). Given that *o*-phthalic acid lacks specificity and is a common metabolite of phthalic anhydride and numerous phthalate diesters, EPA considers *o*-phthalic acid to be a poor biomarker of exposure to phthalic anhydride in cases where there is no known exposure to phthalic anhydride. Therefore, EPA did not further consider epidemiologic studies of *o*-phthalic acid—unless being measured in a population with known exposure to *o*-phthalic acid or phthalic anhydride (*e.g.,* in workers occupationally exposed to phthalic anhydride or *o*-phthalic acid).

2.2 Problem Formulation and Focus of Analysis

Because phthalic anhydride hydrolyzes to *o*-phthalic acid in the environment, the hazard assessment included *o*-phthalic acid to better characterize general population risk in the risk evaluation (U.S. EPA, 2026i). Hazards of *o*-phthalic acid were not relevant to the consumer or occupational risk characterizations because phthalic anhydride products are produced in the absence of water and/or are described as oil-based. Therefore, EPA characterized the hazards of *o*-phthalic acid to derive risk estimates to the general population and characterized the hazards of phthalic anhydride to derive risk estimates to consumers and workers.

Over the past several decades, the human health effects of phthalic anhydride have been evaluated by several regulatory and authoritative agencies, including EPA's Integrated Risk Information System (IRIS) Program (U.S. EPA, 1988), California Office of Environmental Health Hazard Assessment (CalEPA, 2008), Organization for Economic Co-operation and Development (OECD, 2005), Australia National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2013), Health Canada (2019), and the American Conference of Government Industrial Hygienists (ACGIH, 2025). Additionally, *o*-phthalic acid has been evaluated by EPA's Provisional Peer-Reviewed Toxicity Value (PPRTV) Program (U.S. EPA, 2005b). However, no oral or inhalation PPRTV values were derived for *o*-phthalic acid due to the absence of suitable subchronic or chronic toxicity studies via the oral or inhalation exposure routes. Toxicity values from these assessments are shown in Table 2-1. Although EPA incorporated information published in these existing assessments as a starting point for its human health hazard assessment of phthalic anhydride and *o*-phthalic acid, EPA also leveraged novel computational-based NAMs and AOP frameworks, where applicable, for the dermal and inhalation assessments, as described below.

Collaboration of EPA with various organizations including the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), Physicians Committee for Responsible Medicine (PCRM), Inotiv, and *in vitro* method developers (*e.g.,* Invitrolize) facilitated the inclusion of cutting-edge science for this draft assessment consistent with EPAs statutory mandate to include the best available science. For example, over the last several years, NICEATM and ICCVAM have been evaluating the GARDair assay for its ability to identify potential respiratory sensitizers. As part of this inter-agency effort, phthalic anhydride and *o*-phthalic acid were tested in the GARDair (Section 4.3) and GARDskin (Section 4.2) assay. EPA also collaborated with Invitrolize, the developer of an *in vitro* model for evaluating chemicals for respiratory sensitization, for interpretation and testing of phthalic anhydride and *o*-phthalic acid in the ALIsens assay (Section 4.3). Additionally, EPA collaborated with NICEATM and Inotiv for assistance with running and interpreting the results of the Skin Allergy Risk Assessment – Integrated Chemical Environment (SARA-ICE) model, a computational NAMs based approach for dermal point of departure (POD) derivation that consolidates *in chemico*, *in vitro*, and *in vivo* data relevant to an AOP for skin sensitization, consistent with OECD Test Guideline (TG) No. 497 (OECD, 2025b) (Section 4.2).

Existing assessments have consistently identified dermal and respiratory sensitization as the most sensitive hazards associated with dermal and inhalation exposure to phthalic anhydride, respectively. Further, assessments by OECD (2005), Australia NICNAS (2013), and Health Canada (2019) have concluded that phthalic anhydride has low systemic toxicity via the oral exposure route. In addition to these assessments, phthalic anhydride is also classified (GHS) in the EU as Acute Tox. 4 (H302: Harmful if swallowed), Eye Dam. 1 (H318: Causes serious eye damage), Skin Irrit. 2 (H315: Causes skin irritation), Skin Sens. 1 (H317: May cause an allergic skin reaction), Resp. Sens. 1 (H344: May cause allergy or asthma symptoms or breathing difficulties if inhaled), and STOT SE 3 (H335: May cause respiratory irritation) (https://chem.echa.europa.eu/100.001.461/overview?searchText=85-44-9; accessed February 2, 2026).

As discussed in the *Draft Risk Evaluation for Phthalic Anhydride* (U.S. EPA, 2026i), EPA is quantitatively assessing dermal and inhalation exposures to phthalic anhydride for workers and consumers. Oral exposures to phthalic anhydride are not relevant for consumer uses given that the intended use of the products identified (see *Draft Consumer and Indoor Exposure Assessment for Phthalic Anhydride* (U.S. EPA, 2026a)), and oral exposure is not an anticipated route of exposure for workers (See *Draft Environmental Release and Occupational Exposure Assessment for Phthalic Anhydride* (U.S. EPA, 2026g)). The most sensitive hazards identified in existing assessments of phthalic anhydride for the dermal and inhalation exposure routes are dermal and respiratory sensitization. EPA identified no new information that would lead to different conclusions than those reached in existing assessments of phthalic anhydride; therefore, EPA's hazard and dose-response assessment for the dermal and inhalation exposure routes in Sections 4.2 and 4.3 focus on dermal and respiratory sensitization.

For the general population, EPA is evaluating oral, dermal, and inhalation exposures from TSCA releases of phthalic anhydride. Given that phthalic anhydride rapidly hydrolyzes to *o*-phthalic acid in the presence of moisture (*i.e.,* hydrolysis t½ = 30–90 seconds, with complete hydrolysis after ≈8 minutes), the general population is expected to be exposed to *o*-phthalic acid—not phthalic anhydride—from TSCA releases. Notably, unlike phthalic anhydride, *o*-phthalic acid is not a dermal or respiratory sensitizer. Therefore, in support of its general population risk assessment, EPA reviewed all reasonably available information to support hazard identification and dose-response assessment of *o*-phthalic acid. As described in 2.1, epidemiologic studies of *o*-phthalic acid were included if measured in a population

with known exposure to *o*-phthalic acid or phthalic anhydride (*e.g.,* in workers occupationally exposed to phthalic anhydride or *o*-phthalic acid). Reasonably available information was primarily limited to oral exposures studies, which are summarized in Section 4.1.

Additional hazards that were identified in existing assessments but not further considered in this assessment include skin and eye irritation, as well as acute lethality. As mentioned previously, phthalic anhydride is classified (GHS) in the EU as Acute Tox. 4 (H302: Harmful if swallowed), Eye Dam. 1 (H318: Causes serious eye damage), and Skin Irrit. 2 (H315: Causes skin irritation). Original copies of studies supporting these hazards that are cited in existing assessments by OECD (2005) are not reasonably available to EPA for independent review, so they are not further discussed in this draft TSD.

673 **Table 2-1. Summary of Non-Cancer PODs for Phthalic Anhydride Selected for Use by Other Regulatory Organizations**
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Brief Study Description	NOAEC/ LOAEC (mg/m^3)	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	(U.S. EPA, 1988)	(Health Canada, 2019)	(OECD, 2005)	(NICNAS, 2013)	(CalEPA, 2008)
B6C3F1 mice (20/sex in control group; 50/sex in treatment groups) fed diets containing 0, 16,346, 32,692 ppm phthalic anhydride for 104 weeks (equivalent to 0, 1,562, 4,250 mg/kg-day) (NCI, 1979)	–	None / 1,562	↑ incidence of lung and kidney lymphocytosis (both sexes), adrenal atrophy (males only), mineralization of thalamus (males only)	✓[a]		✓[c]	✓[c]	
F344 rats (50/sex/group) fed diets containing 0, 7,500, or 15,000 ppm phthalic anhydride for 105 weeks (equivalent to ≈375 and 750 mg/kg-day) (NCI, 1979).	–	500 / 1,000	↓ body weight gain after 13 weeks		✓	✓[c]	✓[c]	
Discontinuous occupational study of 23 resin plant workers exposed to a mean peak concentration of 6.6 mg/m^3 phthalic anhydride across 2 plants for an average of 13.3 years (Nielsen et al., 1991; Nielsen et al., 1988).	None / 6.5–6.6 mg/m^3 (8-hour TWA = 0.4 mg/m^3)	–	Respiratory tract sensitization. Symptoms of rhinitis and/or conjunctivitis, phthalic anhydride - associated bronchial asthma that was potentially correlated with specific serum IgG antibody levels		✓[b]			✓[d]

↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEC = no-observed-adverse-effect concentration; NOAEL = no-observed-adverse-effect level; LOAEC = lowest-observed-adverse-effect concentration; = LOAEL = lowest-observed-adverse-effect level; TWA = time-weighted average.

[a] LOAEL basis of oral reference dose (RfD) derived by the EPA's IRIS Program (U.S. EPA, 1988). Mean received doses of phthalic anhydride in mg/kg-day were calculated using the following equation: phthalic anhydride in diet (ppm) * (food factor) = mean dose in mg/kg-day, where food factor = 0.13 kg food/kg body weight (bw) for mice and 0.05 kg food/kg bw for rats.

[b] Health Canada (2019) selected critical levels of 6.6 mg/m^3 representative of mean peak exposure concentration associated with respiratory effects in humans, and 0.4 mg/m^3 as the TWA concentration over one day.

[c] OECD (2005) and Australia NICNAS (2013) did not conduct a quantitative human health risk assessment of phthalic anhydride, however, the listed NOAEL and LOAELs values were the lowest values identified in reports by both sources.

[d] California EPA (CalEPA, 2008) derived the chronic reference exposure level from a LOAEC of 6.5 mg/m^3 based on mean peak exposure across two plants (6.1 and 6.8 mg/m^3).

3 TOXICOKINETICS

EPA considered the toxicokinetic information on phthalic anhydride as well as its immediate hydrolysis product, *o*-phthalic acid, as hydrolysis occurs as part of metabolism in a living organism in the presence of water. Identified toxicokinetic information following oral, inhalation, and dermal exposure to phthalic anhydride and/or *o*-phthalic acid is summarized in Sections 3.1 (Oral), Section 3.2 (Inhalation), and Section 3.3 (Dermal). Importantly, *o*-phthalic acid is also a metabolite of phthalate diester chemicals, and therefore is not a specific urinary metabolite of phthalic anhydride or *o*-phthalic acid.

3.1 Oral Route

EPA identified two studies of rats that evaluated absorption, distribution, metabolism, and/or excretion (ADME) of *o*-phthalic acid following oral exposure (Lim et al., 2007; Williams and Blanchfield, 1974). Studies reporting ADME data following oral exposure to phthalic anhydride were not identified.

Lim et al. (2007) investigated the oral toxicokinetics of *o*-phthalic acid. Male Sprague-Dawley (SD) rats (3/dose) were administered single doses of 20, 100, or 500 mg/kg *o*-phthalic acid by gavage in corn oil. Plasma and pooled urine samples were collected at various intervals (plasma intervals: 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dosing; pooled urine intervals: 0−4, 4−8, 8−12, 12−16, and 16−24 hours post-dosing). Levels of *o*-phthalic acid were not evaluated in organs or feces in this study. Peak plasma concentrations of *o*-phthalic acid were achieved 30 minutes post-dosing, indicating rapid absorption of *o*-phthalic acid after oral administration. Peak serum concentrations (C_{max}) were 4.9, 15.9, and 20.5 µg/mL and area under the concentration-time curve (AUC) values were 44.7, 107.6, and 146.9 µg-h/mL for the 20, 100, and 500 mg/kg dose groups, respectively. Terminal plasma elimination halftime values were 5.5, 5.2, and 5.1 hours for the 20, 100, and 500 mg/kg dose groups, respectively. Approximately 13 to 26% of *o*-phthalic acid was excreted unchanged in urine 24 hours post-dosing. Samples were not monitored for other *o*-phthalic acid metabolites, and the total percentage of excreted *o*-phthalic acid could not be determined.

Williams et al. (1974) investigated the toxicokinetics of orally administered *o*-phthalic acid in rats. Excretion of *o*-phthalic acid in urine and feces was assessed in male Wistar rats intubated with a single-dose of radiolabeled *o*-phthalic acid (^{14}C-phthalic acid) at 0.4, 4, and 40 mg/kg (4/dose) and examined at 4, 8, 24, and 48 hours post intubation. Another group of rats intubated with a single dose of 4 mg/kg *o*-phthalic acid was assessed at 4, 8, and 24 hours post intubation (4/dose) for multi-tissue distribution and retention concentration. Nearly 100% ^{14}C-phthalic acid was recovered in urine and feces within 48 hours post exposure for all dose groups. The distribution between urine and feces was consistent across all dose levels and the majority of recovery (≈70–78%) was in feces. However, it is unknown how much of the *o*-phthalic acid excreted in feces was absorbed across the gastrointestinal tract (vs. passed through the gastrointestinal tract unchanged), as biliary excretion was not evaluated in this study. In rats intubated with a single dose of 4 mg/kg, *o*-phthalic acid was detected in spleen, kidney, liver, adipose tissue, skeletal muscle, lungs, testes, heart, and skin. Brain tissue was also examined, but *o*-phthalic acid was not detected. Tissues with the most concentrated distribution at the 4-hour timepoint were spleen, kidney, adipose, and testes; appreciably lower concentrations were detected in these organs at 8 hours post exposure. No radiolabeled *o*-phthalic acid was detected in any organs at 24 hours after dosing.

Based on available data, *o*-phthalic acid is readily absorbed following oral exposure and most of the administered dose is eliminated in urine and feces within 48 hours in rats. EPA assumed an oral absorption of 100%.

3.2 Inhalation Route

EPA identified one study that provided ADME data following inhalation exposure to phthalic anhydride (Pfaffli, 1986). Studies reporting ADME data were not identified for *o*-phthalic acid inhalation exposure.

Pfaffli (1986) investigated the urinary excretion of *o*-phthalic acid in nine workers occupationally exposed to phthalic anhydride in a plant that manufactured phthalic anhydride and unsaturated polyester resins with phthalic anhydride as the starting material. Urine samples were collected pre-shift, on-shift, and post-shift in the evening and the morning following shift work. Urine samples were also collected from 22 workers occupationally unexposed to phthalic anhydride. Inhalation exposure was simultaneously monitored for each worker using a personal breathing zone monitor. Workers were exposed to 8-hour time-weighted average (TWA) atmospheric concentrations of 0.03 to 10.5 mg/m^3 phthalic anhydride. A subset of urine samples were treated with acid hydrolysis, hydrolysis with β-glucuronidase, or alkaline hydrolysis to determine if *o*-phthalic acid was conjugated or excreted as a free acid; however, hydrolysis reactions did not provide any results significantly different from results following immediate *o*-phthalic acid extraction, indicating that *o*-phthalic acid is mainly excreted in urine as the free acid.

The mean concentration of unexposed workers was 0.34 ± 0.25 µmol/mmol creatinine (range: 0.02–0.89 µmol/mmol creatinine, n = 22), and the authors note that *o*-phthalic acid encountered in the urine of unexposed humans may be the product of metabolic hydrolysis of the phthalate diesters. For workers exposed to low atmospheric concentrations of phthalic anhydride (mean: 0.15 ± 0.15 mg/m^3, range: 0.03−0.33 mg/m^3, n = 5), pre-shift (7:00 am), urinary concentrations (0.49 ± 0.15 µmol/mmol creatinine, n = 5) increased until the end of shift (3:00 pm). The authors did not provide the data for the post-shift values, but did provide results of a linear regression analysis, which indicate post-shift (3:00 pm) levels were approximately 1.17 µmol/mmol creatinine. Urinary levels then decreased post-shift until pre-shift urinary concentrations were achieved the following morning (≈0.49 µmol/mmol creatinine), indicating that *o*-phthalic acid was cleared overnight. For workers exposed to higher atmospheric concentrations of phthalic anhydride (mean:1.63 ± 0.13 mg/m^3, n = 3), pre-shift urinary concentrations of *o*-phthalic acid (1.02 ± 0.25 µmol/mmol creatinine, n = 3) were 3 times higher than unexposed workers (0.34 ± 0.25 µmol/mmol creatinine, n = 22), while pre-shift urinary concentrations (4.8 µmol/mmol creatinine) for one worker exposed to the highest atmospheric concentration of phthalic anhydride (*i.e.,* 10.5 mg/m^3) was approximately 14 times that of the control. These results indicate that exposure to higher atmospheric concentrations of phthalic anhydride may result in a body burden of *o*-phthalic acid that cannot be completely cleared overnight. An elimination half-life of 14 hours was reported; however, details regarding how this half-life was calculated were not provided. Although urinary concentrations of *o*-phthalic acid correlated with measured atmospheric concentrations of phthalic anhydride, it is noteworthy that *o*-phthalic acid is a common urinary metabolite of phthalate diesters, including DEHP, DIDP, DBP, DIBP, DINP, DCHP, and BBP.

3.3 Dermal Route

The ADME properties of *o*-phthalic acid have been investigated in one study by the National Toxicology Program (NTP, 1992), but no studies of phthalic anhydride were identified.

NTP (1992) designed a study to evaluate the ADME properties of o-phthalic acid, where F344/N male rats (2−3/dose) were administered single dermal doses of 7.9 or 79 mg/kg radiolabeled o-phthalic acid (14C-U-phthalyl; vehicle = ethanol) and covered with a perforated cap for 7 days. A third group of rats was administered a single dermal dose of 79 mg/kg radiolabeled o-phthalic acid (2 rats), and the dose sites left uncovered for 7 days until sacrifice. Excretion of radiolabeled *o*-phthalic acid in urine and feces

was monitored at 24-hour intervals across the 7-day exposure period for the rats in the capped treatment groups, while tissues (*i.e.,* brain, lung, liver, spleen, small intestine, kidney, testis, fat, muscle, skin, spinal cord, blood, skin at application site, plastic cap) were collected at the end of the 7-day exposure window to determine tissue levels of radiolabeled *o*-phthalic acid in all treatment groups. In the low dose group, approximately 11.3 and 5.8% of administered *o*-phthalic acid was excreted in urine and feces, respectively, after 7 days. In the high dose group, approximately 29 and 22.9% of administered *o*-phthalic acid was excreted in urine and feces, respectively, after 7 days. Approximately 61, 17.6, and 1.8% of administered *o*-phthalic acid was recovered in the skin at the area of application in the low, high (capped), and high (uncapped) dose groups, respectively. Levels of recovered *o*-phthalic acid were highest in the muscle (0.84% [low dose], 0.23% [high dose with cap], 0.15% [high dose uncapped]) and skin (0.63% [low dose], 0.003% [high dose with cap], 0.11% [high dose uncapped]), while the level of recovered *o*-phthalic acid was less than 0.1% in all other examined tissues. Low amounts were recovered in the plastic cap of the low dose group (≈1%) and high dose groups (≈2.7%), and total recovery (sum of dose in urine, feces, tissues, and plastic cap after 7 days of exposure) for low, high dose (capped), and high dose (uncapped) groups was 81, 73, and 98%, respectively. This study indicates that under occluded conditions with an ethanol vehicle, cumulative absorption ranged from low (≈17% at the low dose) to appreciable (≈52%) at the high dose), while systemic retention was minimal.

Available data from one study indicate that dermal absorption of *o*-phthalic acid occurs under some conditions in rats. However, *o*-phthalic acid is largely ionized at a dermal pH in both humans and rats and therefore dermal permeability is expected to be low relative to the un-ionized form in both species due to the influence of ionization on transdermal delivery (OECD, 2022a). As described in the *IH SkinPerm Modeling Manual*, typical skin pH in humans can be up to 5.5 (AIHA, 2017). At these pH values, the first carboxyl group ($pKa_1 = 2.91$) is fully dissociated and the second ($pKa_2 = 5.39$) is partially dissociated, based on the physical-chemical properties of *o*-phthalic acid (U.S. EPA, 2026h). Additionally, as further discussed in the general population screening assessment, EPA did not derive a chronic dermal hazard value for *o*-phthalic acid for use in the general population screening assessment (U.S. EPA, 2026f), which considered incidental dermal exposures due to swimming qualitatively.

For phthalic anhydride, dermal absorption estimates are not needed because the dermal POD based on skin sensitization was derived in terms of an applied dose (see Section 4.2).

4 NON-CANCER HAZARD IDENTIFICATION

This section summarizes the non-cancer hazards associated with oral (Section 4.1), dermal (Section 4.2), and inhalation (Section 4.3) exposure to phthalic anhydride and *o*-phthalic acid. This section focuses on the primary human health hazards for each route of exposure. Summaries of available studies are found in Appendix A. Phthalic anhydride exhibits route-specific toxicity and therefore EPA considered oral, dermal, and inhalation routes separately in the following sections. Where there is evidence that the non-cancer hazards for phthalic anhydride and *o*-phthalic acid differ, they are discussed separately (*i.e.,* hazards via dermal and inhalation routes of exposure). As discussed below, phthalic anhydride is a potent dermal and respiratory sensitizer. However, the available evidence does not support that *o*-phthalic acid is a sensitizer. Target organ toxicity has not been consistently observed following oral exposure to phthalic anhydride or *o*-phthalic acid—both of which have low systemic toxicity through the oral route and are therefore discussed together.

4.1 Oral Exposure

This section summarizes the health effects of phthalic anhydride and *o*-phthalic acid following oral exposure, including health effects observed in humans (Section 4.1.1) and experimental animal models (Section 4.1.3). Mechanistic evidence is summarized in Section 4.1.2. EPA considered studies that report the test substance as phthalic anhydride or *o*-phthalic acid but assumed phthalic anhydride administered via the oral route is hydrolyzed to *o*-phthalic acid within minutes. EPA's evidence integration conclusions, dose-response assessment, and weight of scientific evidence conclusions for the oral route are provided in Sections 4.1.4, 4.1.5, and 4.1.6, respectively.

4.1.1 Summary of Human Evidence

EPA identified several epidemiology studies evaluating associations between urinary, serum, plasma, or semen *o*-phthalic acid levels and various health outcomes (Sol et al., 2020; Philips et al., 2019; Philips et al., 2018; Choi et al., 2014; Song et al., 2014; Sun et al., 2014; Jung et al., 2013; Choi et al., 2012; Miodovnik et al., 2011; Han et al., 2009; Jönsson et al., 2005; Mettang et al., 1996). *o*-Phthalic acid is a common metabolite of phthalic anhydride and many phthalate diesters (*e.g.,* DEHP, DBP, BBP, DIBP, DCHP, DINP, DIDP, etc.), many of which humans are exposed to on a regular basis. Given the lack of specificity, *o*-phthalic acid is a poor biomarker of exposure to phthalic anhydride. Therefore, EPA did not further consider studies of *o*-phthalic acid because none of the populations evaluated in these identified studies have known exposures to phthalic anhydride or *o*-phthalic acid.

4.1.2 Mechanistic Evidence

EPA identified limited mechanistic evidence for phthalic anhydride and *o*-phthalic acid. Using EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/), EPA reviewed available *in vitro* Toxicity Forecasting (ToxCast) data for phthalic anhydride and *o*-phthalic acid, both of which have been tested in 218 *in vitro* assays mapped to various biological targets (accessed March 13, 2025). Phthalic anhydride and *o*-phthalic acid were both inactive in all 218 assays.

EPA further reviewed the mechanistic targets of the available *in vitro* assays using NTP's Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/), which mapped the 218 available assays to 27 mechanistic targets (accessed March 13, 2025). As shown in Table 4-1, mechanistic targets assessed in these *in vitro* assays include numerous nuclear receptors (*e.g.,* androgen, estrogen, glucocorticoid, steroid hormone, thyroid hormone receptors), cell viability processes, energy homeostasis processes, and various stress response pathways (*e.g.,* inflammation, oxidative stress, DNA damage response pathways).

Lack of bioactivity of both phthalic anhydride and *o*-phthalic acid across all tested assays is consistent with results from repeated-dose oral exposure discussed in Section 4.1.3, and supports the conclusion that phthalic anhydride and *o*-phthalic acid have low potential for systemic toxicity via the oral route. It is likely that phthalic anhydride undergoes hydrolysis to *o*-phthalic acid in the aqueous conditions used for the *in vitro* assays.

Table 4-1. Summary of Mechanistic Targets of *In Vitro* ToxCast Assays for Phthalic Anhydride and *o*-Phthalic Acid Identified in the Integrated Chemical Environment

Mechanistic Target	Phthalic Anhydride[a]	*o*-Phthalic Acid[a]
Androgen Receptor Activity	0/6	0/6
Androgen Receptor Activity, Regulation of Cell Cycle	0/1	0/1
Aromatase Activity, Cytochrome P450 Activity	0/1	0/1
Aryl Hydrocarbon Receptor Activity	0/1	0/1
Cell Viability Process	0/77	0/77
Cellular Response to Oxidative Stress, Regulation of DNA-binding Transcription Factor Activity	0/3	0/3
Cellular Response to Stress, Regulation of DNA-binding Transcription Factor Activity	0/1	0/1
Constitutive Androstane Receptor Activation	0/2	0/2
DNA Damage Response	0/4	0/4
DNA Damage Response, Histone Modification	0/1	0/1
Energy Homeostasis	0/3	0/3
Estrogen Receptor Activity	0/8	0/8
Farnesoid X Receptor Activity	0/2	0/2
Glucocorticoid Receptor Activity	0/2	0/2
Histone Modification	0/1	0/1
p53 Signaling Pathway	0/5	0/5
Peroxisome Proliferator Activated Receptor Signaling Pathway	0/4	0/4
Progesterone Receptor Signaling Pathway	0/2	0/2
Regulation of DNA-binding Transcription Factor Activity	0/6	0/6
Regulation of DNA-binding Transcription Factor Activity, Regulation of Inflammatory Response	0/1	0/1
Regulation of Inflammatory Response	0/2	0/2
Retinoic Acid Receptor Activity	0/2	0/2
Retinoid X Receptor Activation	0/1	0/1
Steroid Hormone Receptor Activity	0/4	0/4
Thyroid Hormone Receptor Activity	0/2	0/2
Thyrotropin-Releasing Hormone Receptor Activity	0/4	0/4
Vitamin D Receptor Activity	0/2	0/2

[a] Number of active assays divided by the total number of assays mapped to the mechanistic target; data accessed from NTP's Integrated Chemical Environment (ICE) (https://ice.ntp.niehs.nih.gov/) on March 13, 2025.

4.1.3 Evidence Integration Summary of Laboratory Animal Evidence

Phthalic anhydride and *o*-phthalic acid have been evaluated in a number of oral exposure studies of mice and rats. Available studies of phthalic anhydride include: two subchronic (>30–90 days) dietary dose-range finding studies (1 each of mice and rats) (NCI, 1979); two chronic (>90 days) dietary studies (1 each of mice and rats) (NCI, 1979); and one teratogen screening study of mice (Fabro et al., 1982). Available studies of *o*-phthalic acid include: four intermediate duration (>1–30 days) studies of rats (Kwack et al., 2010; Kwack et al., 2009; Oishi and Hiraga, 1980; Lake et al., 1975); one subchronic study of rats (Murakami et al., 1986); two developmental studies of rats (Rahmani et al., 2015; Ema et al., 1997); and one sperm head abnormality assay with mice (Jha et al., 1998). Available oral studies are summarized in Table 4-2 and Table 4-3; study summaries are provided in Appendix A.

Systemic effects following repeated oral exposure to phthalic anhydride and *o*-phthalic acid are discussed in Section 4.1.3.1; developmental and reproductive effects of phthalic anhydride and *o*-phthalic acid are discussed in Section 4.1.3.2.

4.1.3.1 Systemic Effects

Available oral exposure studies indicate that both phthalic anhydride and *o*-phthalic acid have low systemic toxicity following exposure via gavage and/or dietary exposures. NCI (1979) reports results of two 7-week dietary dose-range finding studies (1 of mice, 1 of rats) and 2-year dietary studies of phthalic anhydride (1 of mice, 1 of rats) (Table 4-2). In the first dose-range finding study, male and female F344 rats were fed diets containing 0, 6,200, 12,500, 25,000, or 50,000 ppm phthalic anhydride (equivalent to ≈230, 463, 926, 1,853 mg/kg-day) for 7 weeks. No effect on survival was observed, while terminal bodyweight was reduced in high-dose male and female rats by 24 to 26% at study week 7. Histopathologic findings were limited to the livers of male rats in the 25,000 ppm group (trace amounts of centrilobular cytoplasmic vacuolation was observed in 4 out of 5 males); however, the effect was not dose-dependent, as tissues were normal in high-dose (50,000 ppm) male and female rats. Overall, EPA considered this study to support a NOAEL of 926 mg/kg-day based on reduced terminal body weight in high-dose male and female rats. In the subsequent 2-year bioassay, male and female F344 rats were fed diets containing 0, 7,500, or 15,000 ppm phthalic anhydride (equivalent to ≈278 or 556 mg/kg-day) for 105 weeks. Observed effects were limited to a reduction in body weight gain from study week 13 to the end of the study for high-dose males (but not females). However, quantitative measures of the average weights and variances were not provided, so the magnitude of the effect on body weight gain was estimated from growth curves by EPA. The effect appeared to be approximately 10% at most timepoints. No effects on survival or incidence of any non-neoplastic or neoplastic lesions in any organ were observed. Other outcomes (*i.e.,* organ weight, hematology, clinical chemistry, food consumption) were not evaluated.

In the second dose-range finding study, male and female B6C3F1 mice were fed diets containing 0, 6,200, 12,500, 25,000, or 50,000 ppm phthalic anhydride (equivalent to ≈692, 1,389, 2,279, 5,558 mg/kg-day) for 7 weeks (NCI, 1979) (Table 4-2). Treatment with phthalic anhydride had no effect on survival or terminal body weight, and no histopathologic findings were reported. In the subsequent 2-year dietary study, male and female B6C3F1 mice were fed diets containing 0, 25,000, or 50,000 ppm phthalic anhydride based on the results of the dose-range finding study. However, due to excessive body weight loss in both sexes, dietary concentrations of phthalic anhydride were reduced to 12,500 and 25,000 ppm for males and 6,250 and 12,500 ppm for females starting on study week 32 (TWA doses ≈1,817 or 3,634 mg/kg-day [males] and 1,336 and 2,672 mg/kg-day [females]). No effects on survival were observed. Dose-related decreases in body weight gain were observed in low- and high-dose mice with terminal body weight reduced by 12 to 27% in low- and high-dose mice of both sexes. No treatment-related neoplastic changes were observed in any tissue or treatment group. NCI (1979)

concluded that there were no treatment-related non-neoplastic pathological effects in either male or female mice. However, U.S. EPA (1988) IRIS program re-evaluated histopathology data and found significant increases in the incidence of several non-neoplastic lesions— including lung and kidney lymphocytosis in males and females (both dose groups); mineralization of the thalamus of males (both dose groups); atrophy of the adrenal cortex of males (both dose groups); and bile duct inflammation of males and females (high-dose only) (incidence data provided in Table_Apx A-1).

The 2-year studies by NCI (1979) support a NOAEL of 278 mg/kg-day, based on reduced body weight gain in male rats and a LOAEL (no NOAEL identified) of 1,336 mg/kg-day for based on histologic findings and reduced body weight in female mice. However, these studies reported issues with the stability of phthalic anhydride in the dosed feed mixtures. NCI (1979) states, "Assays of the dosed feed mixtures indicated that they may have been unstable under the conditions of use." This is based on a stability analysis of feed mixtures containing 15,000 ppm phthalic anhydride that lost 2.59% (or 372 ppm) per day when stored at room temperature. From the analysis, it is unclear if phthalic anhydride was lost due to hydrolysis to *o*-phthalic acid or covalent interactions of phthalic anhydride with proteins in the feed that may have limited the availability of the test substance. Loss is not expected to have been due to volatilization, as phthalic anhydride has low volatility (*i.e.,* vapor pressure of 5.14×10^{-4} mmHg, see (U.S. EPA, 2026h)). NCI (1979) states that feed mixtures were prepared fresh every one-to-one and half weeks and diet was routinely stored at 5 °C until its use. Storage of the diet at 5 °C may have slowed phthalic anhydride loss; however, stability was not assessed at 5 °C. The identified stability issues contribute uncertainty regarding the precise doses of phthalic anhydride received by animals in these studies. Nevertheless, the authors reported information was sufficient for EPA to estimate the received doses, which were sufficient to elicit effects (primarily on body weight) in mice and rats. Assuming chemical loss per day of 2.59% and that food was freshly prepared every 10 days, EPA estimated the received doses to account for 26% loss of test substance; the adjusted doses accounting for this loss are shown below in Table 4-2. These studies were considered quantitatively for dose-response assessment using the adjusted doses (Section 4.1.5).

The *o*-phthalic acid oral exposure studies identified by EPA evaluated effects following gavage (Kwack et al., 2010; Kwack et al., 2009; Lake et al., 1975) or dietary (Murakami et al., 1986; Oishi and Hiraga, 1980) exposures.

Lake et al. (1975), gavaged male Wistar rats with 0 or 850 mg/kg-day *o*-phthalic acid for 7 days (Lake et al., 1975), and no effects on any liver outcomes were observed (*i.e.,* absolute/relative liver weight, histopathology, biochemical parameters; Table 4-2). However, the scope of that study was limited to evaluation of liver outcomes and the study authors did not report body weight.

Consistent reductions in terminal body weight were observed in 14- and 28-day studies of *o*-phthalic acid in the absence of effects on food consumption ((Kwack et al., 2010); Table 4-2). Kwack et al. gavaged male SD rats with 0 or 250 mg/kg-day *o*-phthalic acid for 14 (Kwack et al., 2010) or 28 days (Kwack et al., 2009) and reported 14 and 22% reductions in mean terminal bodyweight, respectively. No effects on survival, food consumption, relative organ weight (*i.e.,* liver, kidney, testis, epididymis, adrenal, spleen, heart, lung, thyroid, and thymus), hematology, clinical chemistry, or urinalysis parameters were observed in either study. A 33% reduction in curvilinear sperm velocity was observed in the 28-day study; however, no other sperm parameters were altered (*i.e.,* count, motility, other measures of velocity) (Kwack et al., 2009). Both studies have several limitations, including small sample size (n = 6 per group), evaluation of only male rats, and lack of histopathologic examination.

Two dietary studies were available that provided information on systemic health effects following oral exposure to *o*-phthalic acid. In a dietary study by Oishi et al. (1980), male Wistar rats were fed diets containing 0 or 2% *o*-phthalic acid (equivalent to 2,000 mg/kg-day) and the only observed effect was a slight increase (11%) in measured levels of zinc in the kidney (Oishi and Hiraga, 1980). No effects on bodyweight gain; absolute/relative testes, liver, or kidney weight; testicular or serum testosterone; or levels of zinc in the testes, liver or serum were observed (Table 4-2). In another dietary study, male Wistar rats were fed diets containing 0, 0.5, and 5.0% *o*-phthalic acid (equivalent to 500 and 5,000 mg/kg-day) for 34 to 36 days (Murakami et al., 1986) (Table 4-2). No effects on body weight gain; absolute/relative liver, kidney, spleen, and testes weight; serum chemistry parameters; or liver, kidney, and testis histopathology were observed at any dose. Limitations of this study include small sample size (n = 5 per group), evaluation of only male rats, and evaluation of a limited number of organs.

Both phthalic anhydride and *o*-phthalic acid are considered irritants. Therefore, EPA also considered whether reductions in body weight gain and terminal body weight observed in oral studies of rats and mice may be secondary to portal-of-entry effects in the gastrointestinal tract as a potential mode of action (MOA). It is plausible that irritation, inflammation, or other types of tissue damage in the stomach or intestines may lead to nausea and subsequent decreases in food consumption and weight gain. However, available studies reporting reduced body weight gain or terminal body weight either do not evaluate food consumption (*e.g.,* studies by NCI (1979)) or report inconsistent effects on food consumption. Available evidence, when considered together, does not provide sufficient support for this MOA. NCI (1979) reported microscopic evaluations of the stomach, and small and large intestines in male and female rats and mice exposed to phthalic anhydride in the diet for 2 years and observed no treatment-related findings at doses that significantly reduced body weight gain. No effects on food consumption were reported in 14- and 28-day studies in which rats were gavaged with 250 mg/kg-day *o*-phthalic acid (Kwack et al., 2010; Kwack et al., 2009), while reduced food consumption coincided with reduced maternal weight gain at very high doses (1,763 to 2,981 mg/kg-day) of *o*-phthalic acid in a prenatal study of rats (see Section 4.1.3.2) (Ema et al., 1997).

Although the database of oral exposure studies has limitations, available studies consistently indicate that phthalic anhydride and *o*-phthalic acid have low systemic toxicity by the oral route, with the most consistently observed effects being reduced body weight gain and terminal body weight. Consistent effects on specific target organs have not been observed across available studies. Notably, EPA's draft conclusion is consistent with the conclusions of OECD (2005), Health Canada (2019), and Australia NICNAS (2013) who also concluded phthalic anhydride has low systemic toxicity by the oral route.

982 **Table 4-2. Summary of Oral Exposures Studies of Phthalic Anhydride and *o*-Phthalic Acid[a]**

Brief Study Description	Study Quality Rating[b]	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Studies of phthalic anhydride				
F344 rats (5/sex/dose) fed diets containing 0, 6,200, 12,500, 25,000, or 50,000 ppm phthalic anhydride for 7 weeks (equivalent to 230, 463, 926, 1853 mg/kg-day) followed by a 1-week observation period (NCI, 1979) [c d]	Medium	926 / 1,853	24−26% ↓ terminal body weight (both sexes)	- ↓ terminal body weight at 50,000 ppm (both sexes) (male % control: 90, 95, 92, 74%; female % control: 95, 93, 91, 76%) - Centrilobular cytoplasmic vacuolation in liver of 4 males at 25,000 ppm; however, tissues normal at 50,000 ppm for both sexes Unaffected outcomes - Survival; histopathology (females) Considerations - Food consumption not reported - Dose range finding study to determine MTD for subsequent 2-year study - Organ weight, clinical chemistry, hematology not evaluated - Potential issues with stability of test substance in diet, which reduced confidence in estimated received doses
B6C3F1 mice (5/sex/dose) fed diets containing 0, 6,200, 12,500, 25,000, or 50,000 ppm phthalic anhydride for 7 weeks (equivalent to 692, 1,389, 2,779, 5,558 mg/kg-day) followed by 1-week observation period (NCI, 1979) [c d]	Medium	5,558/ None	None	Unaffected outcomes - Survival; terminal body weight; histopathology Considerations - Food consumption not reported - Dose range finding study to determine MTD for subsequent 2-year study - Organ weight, clinical chemistry, hematology not evaluated - Potential issues with stability of test substance in diet, which reduced confidence in estimated received doses
B6C3F1 mice (20/sex in control group; 50/sex in dose groups) fed diets containing 0, 16,346 and 32,692 ppm phthalic anhydride for 104 weeks (equivalent to 1,817 and 3,634 mg/kg-day) for males and 12,019 and 24,038 ppm (≈1,336 and 2,672 mg/kg-day) for females (NCI, 1979) [c d]	Medium	None/ 1,817 (males) None/ 1,336 (females)	↓ terminal body weight (12−27%) (both sexes) ↑ incidence of lung and kidney lymphocytosis (both sexes) and ↑ incidence of atrophy of adrenal cortex and	Affected Outcomes - ↓ body weight gain and terminal body weight (12−27% ↓ in both sexes of both dose groups) Histopathological Findings - ↑ Lung lymphocytosis (both sexes; both dose groups) - ↑ Kidney lymphocytosis (both sexes; both dose groups) - ↑ Bile duct inflammation (both sexes; high-dose only) - ↑ Atrophy of adrenal cortex (males only; both dose groups) - ↑ Mineralization of thalamus (males only; both dose groups) Unaffected outcomes:

Brief Study Description	Study Quality Rating[b]	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			mineralization of thalamus (males only)	- Survival Considerations: - Food consumption not reported - Organ weight, serum chemistry, hematology not evaluated - Evidence of overt toxicity (excessive body weight loss) after 32 weeks of study leading study authors to reduce dietary concentrations of phthalic anhydride (male doses reduced from 25,000 and 50,000 ppm to 12,500 and 25,000 ppm; females doses reduced to 6,250 and 12,500 ppm for females) - Potential issues with stability of test substance in diet, which reduced confidence in estimated received doses
F344 rats (20/sex in control group; 50/sex in dose groups) fed diets containing 0, 7,500 or 15,000 ppm phthalic anhydride for 105 weeks (equivalent to 0, 278 or 556 mg/kg-day (NCI, 1979) [c d]	Medium	278/ 556	↓ body weight from study week 13 to end of study (males only)	Unaffected outcomes: - Survival, histopathology - Bodyweight gain (females only) Considerations: - Food consumption not reported - Organ weight, serum chemistry, hematology not evaluated - Low incidence of clinical outcomes observed, but reported qualitatively only (*e.g.,* incidences were described as "low" for "arched back, rough hair coat, ulceration, and corneal opacity") - Magnitude of body weight change for high-dose males reported graphically only (depressed ≈10%) - Potential issues with stability of test substance in diet, which reduced confidence in estimated received doses
Studies of *o*-phthalic acid				
Male Wistar rats (6/group) administered 0 (corn oil vehicle) or 850 mg/kg-day *o*-phthalic acid via gastric intubation for 7 days (Lake et al., 1975)	Medium	850/ None	None	Effects at 850 mg/kg-day - None Unaffected Outcomes - Liver weight (relative); liver histopathology; biochemical parameters in liver homogenate (succinate dehydrogenase activity, glucose-6-phosphatase activity, aniline 4-hydroxylase activity, biphenyl 4-hydroxylase activity, cytochrome P-450 content, microsomal protein content) Considerations - Bodyweight not reported - Only evaluated liver outcomes (other organs not evaluated) - Clinical chemistry and hematology not evaluated

Brief Study Description	Study Quality Rating[b]	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Male Wistar rats fed diet containing 0 (n = 20) or 2% (n = 10) *o*-phthalic acid for 7 days (equivalent to 0 or 2,000 mg/kg-day) (Oishi and Hiraga, 1980)[c]	Medium	2,000/ None	None	Effects at 2,000 mg/kg-day - ↑ (11%) levels of Zn in kidneys (not considered adverse) Unaffected Outcomes - Bodyweight gain; absolute or relative weight of the testes, liver, or kidneys; testicular testosterone levels; serum testosterone & dihydrotestosterone levels; levels of Zn in testes, liver, or serum Considerations - Histopathology, clinical chemistry, hematology not evaluated
Male SD rats (6/group) gavaged with 0 (corn oil vehicle) or 250 mg/kg-day *o*-phthalic acid for 14 days (Kwack et al., 2010)	High	None/ 250	↓ mean terminal body weight (14%)[e]	Unaffected outcomes - Survival; food consumption; relative weight of liver, kidney, testis, epididymis, adrenal, spleen, heart, lung, thyroid, thymus; hematological parameters; serum clinical chemistry parameters; urinalysis parameters Considerations - Absolute organ weight not reported - Histopathologic examination not conducted
Male SD rats (6/group) gavaged with 0 or 250 mg/kg-day *o*-phthalic acid for 28 days (Kwack et al., 2009)	High	None/ 250	↓ mean terminal body weight (22%), ↓ curvilinear sperm velocity[f]	Effects at 250 mg/kg-day - Change in sperm parameter (curvilinear velocity; ↓ 33%). Unaffected Outcomes - Survival; food consumption; relative weight of adrenal, heart, kidney, lung, liver, spleen, thymus, thyroid, testis, or epididymis; hematological parameters; serum clinical chemistry parameters; sperm parameters (*e.g.,* sperm count, % motile sperm, additional sperm motility parameters) Considerations - Absolute organ weight not reported - Histopathologic examination not conducted
Male Wistar rats (5/dose) were fed diets containing 0, 0.5, or 5% *o*-phthalic acid for 34–36 days (equiv. 0, 500, 5,000 mg/kg-day) (Murakami et al., 1986) [c]	Medium	5,000/ None	None	Unaffected Outcomes - Body weight gain; absolute or relative weight of the liver, kidneys, spleen, testicles - Serum levels of ALP, GOT, GPT, cholesterol, LDH, globulin levels, lactate dehydrogenase, albumin-to-globulin ratio, albumin, triglycerides, uric acid, creatine phosphokinase - Dehydrogenase activities in liver mitochondria

Brief Study Description	Study Quality Rating[b]	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- Liver, kidney, and testicle histopathology

↓ = statistically significant decrease; ↑ = statistically significant increase; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; MTD = maximum tolerated dose; ALP = alkaline phosphatase; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; LDH = lactate dehydrogenase

[a] Table reflects author reported test articles administered (*i.e.,* phthalic anhydride or *o*-phthalic acid). See Appendix A.1 for detailed summaries of oral exposure studies.

[b] Indicates the study quality rating for the utility of a particular study for quantitative dose-response; does not indicate utility for qualitative weight of scientific evidence.

[c] Mean received doses in mg/kg-day and food consumption were not reported. To estimate the mean received doses of *o*-phthalic acid or phthalic anhydride in mg/kg-day, a food factor was used (% *o*-phthalic acid or phthalic anhydride in diet × food factor × 10,000 = mean dose in mg/kg-day), where food factor = 0.05 for old rats, 0.1 for young rats, and 0.15 for mice (WHO, 1987).

[d] Adjusted doses in mg/kg-day reflect EPA calculations based on author-reported information in (NCI, 1979), which assume loss of phthalic anhydride in feed to be 25.9%.

[e] Percent change estimated from Figure 2 of Kwack et al. (2010).

[f] Percent change estimated from Figure 2b of Kwack et al. (2009).

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4.1.3.2 Developmental and Reproductive Effects

EPA identified four studies that evaluated the reproductive and developmental toxicity of phthalic anhydride or *o*-phthalic acid, including one teratogen screening study of mice exposed to phthalic anhydride (Fabro et al., 1982), two developmental studies of rats exposed to *o*-phthalic acid (Rahmani et al., 2015; Ema et al., 1997), and one sperm head abnormality assay with mice exposed to *o*-phthalic acid (Jha et al., 1998). Available studies are summarized in Table 4-3 and Appendix A.2. Neither phthalic anhydride or *o*-phthalic acid have been evaluated in reasonably available one or two-generation studies of reproduction. Although OECD 414 (Prenatal Developmental Toxicity Study) and 443 (Extended One-Generation Reproductive Toxicity Study) studies of phthalic anhydride are underway in the EU to meet REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulations, the submission deadline for these studies is March 30, 2027,[3] and therefore these studies were not reasonably available to EPA at the time of this assessment.

In a teratogen screening study by Fabro et al. (1982), pregnant CD-1 mice were injected intraperitoneally (i.p.) with phthalic anhydride on gestation days (GDs) 8 through 10 and then sacrificed on GD 18. Study authors did not report the precise number of treatment groups or administered doses. Adult LD01 and LD50 (lethal dose that caused 1 or 50% mortality) values were reported to be 0.37 mmol/kg-day (95% CI: 0.19−0.43 mmol/kg-day) and 0.51 mmol/kg-day (95% CI: 0.44−0.57 mmol/kg-day), respectively. The tD_{05} and tD_{50} values, defined as the "doses require to induce an additional 5 or 50% malformation rate above the background" were extrapolated to be 0.40 and 1.37 mmol/kg-day (95% confidence intervals [CIs] could not be calculated). Teratogenic effects were observed at doses that also caused lethality in non-pregnant female mice (*i.e.,* LD_{01} and tD_{05} overlap). The relevance of these findings is questionable due to poor documentation of administered doses and dose groups, the fact that teratogenicity was observed at doses that also caused maternal lethality, and the human relevance of the route of exposure (i.p. injection). Further, the route of administration is of particular concern for phthalic anhydride, which is an irritant, and may cause portal of entry effects in the peritoneal cavity.

In the first developmental study, pregnant Wistar rats (11/group) were fed diets containing 0, 1.25, 2.5, or 5.0% *o*-phthalic acid (equivalent to 1,021, 1,763, and 2,981 mg/kg-day) on GDs 7 through 16 and then sacrificed on GD 20 (Ema et al., 1997). Dam food consumption was reduced 13 to 27% and dam body weight gain was reduced 18 to 59% at 1,763 mg/kg-day and above on GDs 7 through 16. Maternal weight gain adjusted for gravid uterine weight was reduced 40% at 2,981 mg/kg-day. Developmental effects coincided with maternal toxicity and were limited to a slight (4%) decrease in live male (but not female) fetus weight and a slight (7%) decrease in number of ossification centers at 2,981 mg/kg-day. In rodents, ossification begins in mid-gestation and continues until birth (Desesso and Scialli, 2018). No malformations or skeletal variations were observed at any dose.

In a second developmental study, pregnant Wistar rats (8/group) were fed diets containing 0, 2.5, or 5.0% *o*-phthalic acid (equivalent to 1,763, and 2,981 mg/kg-day) on GDs 7 through 16 (Rahmani et al., 2015). Dams were then allowed to give birth to pups naturally and cardiovascular outcomes were evaluated in three-month-old F1 offspring. In three-month-old F1 offspring, mean body weight was reduced 14 to 25% (≥1,763 mg/kg-day); absolute heart weighted was reduced 12 to 19% (≥1,763 mg/kg-day), while relative heart weight increased 8% (2,981 mg/kg-day); blood pressure increased 5.5 to 12% (≥1,763 mg/kg-day); heart rate increased 8.9% (2,981 mg/kg-day); and various measures of aorta and coronary artery thickness were increased. However, results of this study must be interpreted cautiously,

[3] https://echa.europa.eu/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e185f78d14 (accessed February 2, 2026)

given limitations of the study. First, study authors did not evaluate or report any maternal (survival, food consumption, body weight gain, etc.) or any developmental (# of live/dead offspring per litter, sex ratio, etc.) outcomes. Given that doses were selected for this study based on the study by Ema et al. (1997), it is likely that maternal toxicity occurred in dams of both dose groups. Second, for the majority of evaluated outcomes, study authors did not state whether effects were evaluated in male, female, or combined male and female F1 offspring, which could confound results. Finally, given the reduction in F1 offspring body weight (*i.e.,* 14–25% decrease), it is unclear if the observed cardiovascular effects are directly attributable to *o*-phthalic acid or are secondary to the 14 to 25% reduction in body weight.

Rahmani et al. (2015) was designed to evaluate cardiotoxicity, but the aforementioned limitations of the study impacted the ability to interpret the results. Nevertheless, the consistency across endpoints primarily at 2,981 mg/kg-day, including increased blood pressure and histopathological effects in the aorta suggest that *o*-phthalic acid may be toxic to the cardiovascular system, albeit at very high doses.

Jha et al. (1998) conducted a sperm head abnormality assay. Adult male Swiss albino mice (n = 5/group) were administered a single i.p. injection of 0, 50, 100, 150, 200, or 300 mg/kg *o*-phthalic acid or vehicle (10% DMSO in PBS). Mice were sacrificed 1, 3, and 5 weeks after exposure and then smears of spermatozoa from epididymides were evaluated for abnormal sperm. A statistically significant increase in the incidence of sperm head abnormalities was observed at doses of 100 mg/kg-day and up at 1 and 3 weeks (spermatozoa and spermatid stages). The authors report that the most common types were "amorphous, elongate, without hook, and giant amorphous." At 5 weeks, a significant increase in abnormal sperm was only observed in the high-dose group (*i.e.,* 300 mg/kg-day). However, results from this study must be interpreted with caution, as i.p. injection is not a human-relevant exposure route and as discussed above, this route is of particular concern for irritants such as *o*-phthalic acid, which may cause portal of entry effects.

One limitation of the reasonably available developmental studies is that none evaluated exposures during late gestation (*i.e.,* GD 17 to "as close as possible to the normal day of delivery"), as recommended by current OECD TG 414 (Prenatal Developmental Toxicity Study) (OECD, 2018b). Sensitive windows of exposure for developmental outcomes exist outside of this period, including mineralization of the skeleton. Indeed, the rat amasses up to 95% of the total calcium required for bone mineralization during the last 5 days of its 22-day gestation (ASBMR, 2006; Domingo, 1998). Moreover, some evidence suggests *o*-phthalic acid can act as a nutrient complexing agent (Sorouraddin et al., 2019; OECD, 2001), which can cause several types of toxicity, including developmental toxicity at least in part due to induced trace element deficiencies (Swenerton and Hurley, 1971). Trace element deficiencies manifest after the complexing agent forms complexes with positively charged metals and trace elements via its negatively charged deprotonated carboxylic acid groups (Sorouraddin et al., 2019), as has been demonstrated for terephthalic acid (isomer of *o*-phthalic acid) (OECD, 2001). Therefore, the incomplete coverage of critical windows is a source of uncertainty for phthalic anhydride and *o*-phthalic acid.

Uncertainty related to incomplete coverage of critical windows can be partially addressed by studies of *o*-phthalic acid isomers, including terephthalic acid and isophthalic acid. As discussed by OECD (2001), a one-generation reproduction feeding study in rats exposed to terephthalic acid reported postnatal developmental effects. However, these effects were attributed to maternal toxicity and the formation of renal and bladder calculi in the F1. Furthermore, adverse effects of terephthalic acid following oral exposure are almost completely restricted to the urinary tract and are consistent with terephthalic acid acting as a nutrient complexing agent. Following subchronic and chronic oral exposure to terephthalic acid, observed effects include: formation of renal and bladder calculi/stones (composed primarily of

calcium-terephthalate complex), inflammatory changes and hyperplasia of the bladder epithelium, changes in urinalysis parameters (*e.g.,* hematuria and proteinuria), and formation of bladder tumors (Table 4-4). Similar effects were observed in male and female parental rats and F1 offspring in a one generation study of reproduction of terephthalic acid (OECD, 2001). In contrast, there is less evidence available supporting effects consistent with nutrient complexing for isophthalic acid (Table 4-4). As reported by OECD (2002), formation of bladder calculi and histopathology in the urinary bladder were not observed in a comparative study of terephthalic acid and isophthalic acid in Wistar rats exposed to up to 5.0% isophthalic acid (equivalent to 2,500 mg/kg-day) for 13 weeks. In contrast, Wistar rats exposed to up 2,100 to 2,500 mg/kg-day terephthalic acid developed bladder calculi and hyperplasia. Effects of isophthalic acid were limited to the kidney (*i.e.,* increased incidence of crystalluria, mild hydronephrosis, pelvic calcification) at doses of 800 mg/kg-day isophthalic acid and above.

There is no evidence of urinary bladder calculi/stones, urinary bladder histology, or urinary bladder tumors in 2-year studies of rats at doses as high as 556 mg/kg-day phthalic anhydride or mice at doses as high as 2,672 (females) to 3,634 mg/kg-day (males; Table 4-4) (NCI, 1979). Finally, no evidence of hematuria or proteinuria was observed in male rats gavaged with 250 mg/kg-day *o*-phthalic acid for 14 to 28 days (Kwack et al., 2010; Kwack et al., 2009). Collectively, these results suggest that *o*-phthalic acid may not have the same nutrient complexing properties as terephthalic acid *in vivo.* While it is possible that *o*-phthalic acid may act through another MOA to result in developmental effects, the available data suggest that reproductive and developmental effects of *o*-phthalic acid occur only at doses which elicit maternal toxicity. Therefore, the absence of developmental studies directly informing the effects of late gestational exposures to *o*-phthalic acid is not likely to substantially contribute to scientific uncertainty in the qualitative and quantitative risk assessment for phthalic anhydride and *o*-phthalic acid.

Overall, EPA evaluated the available evidence for developmental and reproductive toxicity following exposure to phthalic anhydride and/or *o*-phthalic acid, which included four studies. Available studies generally did not observe developmental effects at doses below those which elicit maternal toxicity, which themselves were at very high doses (*i.e.,* 1,763 mg/kg-day). One limitation of the database was the lack of coverage of sensitive windows of development during late gestation (*i.e.,* GD 17 to "as close as possible to the normal day of delivery"), which is recommended to include in study designs by current OECD guidelines (OECD, 2018b), and reflects a period in which mineralization of the skeleton occurs. Because of the lack studies directly informing possible effects of late gestational exposures to *o*-phthalic acid, and the evidence that a phthalic acid isomer may act as a nutrient complexing agent, EPA considered the evidence of the nutrient complexing properties of *o*-phthalic acid and two isomers. Ultimately, EPA concluded that the absence of developmental studies directly informing the effects of late gestational exposures to *o*-phthalic acid is unlikely to substantially contribute to scientific uncertainty in the qualitative and quantitative risk assessment for phthalic anhydride and *o*-phthalic acid. EPA concluded that there is no evidence that developmental toxicity occurs at doses below those which elicit maternal toxicity, which is consistent with conclusions of the OECD (2005) and Health Canada (2019). The Agency discusses these conclusions further in the weight of scientific evidence conclusions (Section 4.1.6 below).

Table 4-3. Summary of Developmental Toxicity Studies of *o*-Phthalic Acid or Phthalic Anhydride

Brief Study Description	Study Quality Rating	NOAEL/LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Pregnant Wistar-Kyoto rats (11 dams/group) fed diets containing 0, 1.25, 2.5, or 5.0 % *o*-phthalic acid from GD 7 through GD 16 and sacrificed on GD 20 (equivalent to 0, 1,021, 1,763, 2,981 mg/kg-day) (Ema et al., 1997)	High	1,021/ 1,763 (Maternal) 1,763/ 2,981 (Developmental)	Maternal: ↓ body weight gain (18−59%) and ↓ (13−27%) food consumption (13−27%) from GD7 – GD16 Developmental: ↓ mean body weight (≈4%) of live male fetuses and ↓ (7%) # of ossification centers of caudal vertebrae on GD20	Maternal Effects - ↓ body weight gain (18−59%) from GD7–GD16 (≥1,763 mg/kg-day) - ↓ body weight gain (18−59%) from GD16–GD20 (≥1,763 mg/kg-day) - ↓ (40%) Maternal weight gain adjusted for gravid uterine weight (2,981 mg/kg-day) - ↓ food consumption (13−27%) from GD7–GD16 (≥1,763 mg/kg-day) - ↑ food consumption (11–36%) from GD 16–GD 20 (≥1,021 mg/kg-day) Developmental Effects - ↓ (4%) live male fetus weight (2,981 mg/kg-day) - ↓ (7%) # of ossification centers of caudal vertebrae (2,981 mg/kg-day) Unaffected Outcomes - Mortality (dams); clinical signs (dams); # of litters; # corpora lutea per litter; # implantations per litter; # of total litter resorptions; # dead fetuses per litter; incidence of post-implantation loss; # live fetuses; sex ratio; mean body weight of female fetuses; incidence of external, skeletal and internal malformations Considerations - Fewer dams per dose group than recommended by OECD TG No. 414 - Exposure duration did not include late gestation, as recommended by current OECD TG No. 414
Pregnant Wistar rats (8 dams/group) fed diets containing 0, 2.5, or 5.0% *o*-phthalic acid from GD 7 - GD 16 (equivalent to 0, 1,763, or 2,981 mg/kg-day) (Rahmani et al., 2015)	Medium	None/ 1,763	↓ pup body weight; ↑ systolic blood pressure; ↓ absolute heart weight; ↑ artery thickness	- ↓ (14−25%) F1 offspring body weight at 3 months of age (≥1,763 mg/kg-d) - ↑ (5−12%) blood pressure (≥1,763 mg/kg-d); ↑ (9%) heart rate (2,981 mg/kg-d); ↓ (12−19%) absolute heart weight (≥1,763 mg/kg-d); ↑ (8%) relative heart weight (2,981 mg/kg-d) in F1 offspring at 2 months of age - ↑ wall thickness and cross-sectional area of thoracic aorta and septal branch of coronary artery in F1 offspring at 3 months of age (≥1,763 mg/kg-d) - ↑ malondialdehyde, ↓ superoxide dismutase, ↓ nitric oxide synthesis activity (≥1,763 mg/kg-d) and ↓ glutathione peroxidase activity (2,981 mg/kg-d) in heart tissue from 3-month-old F1 males Considerations - Maternal effects (body weight gain, food consumption, survival) not reported

Brief Study Description	Study Quality Rating	NOAEL/LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- Developmental and reproductive outcomes beyond pup body weight not reported - For most outcomes, authors did not report if measured in male, female, or combined F1 offspring - Fewer dams per dose group than recommended by OECD TG No. 414 - Exposure duration did not include late gestation, as recommended by current OECD TG No. 414
Male Swiss albino mice (5/group) administered single i.p. injections of 0 (10% DMSO in PBS), 50, 100, 150, 200, 300 mg/kg *o*-phthalic acid. Smears of spermatozoa from epididymides evaluated afer 1, 3, and 5 weeks (Jha et al., 1998)	High (though uninformative for dose-response assessment given the method (i.p. injection) of exposure)	NA	NA	- ↑ # of sperm abnormalities at ≥100 mg/kg during weeks 1 and 3 - ↑ # of sperm abnormalities at 300 mg/kg during week 5 Considerations - Effects on food consumption, body weight gain, clinical signs not evaluated - Route of administration (i.p. injection) lacks human relevance
Pregnant CD-1 mice were i.p. injected with phthalic anhydride (0.5% in methylcellulose) on GDs 8 through 10 and then sacrificed on GD 18 (Fabro et al., 1982)	Uninformative	NA	NA	- Adult LD01 and LD50 values were reported to be 0.37 mmol/kg-day (95% CI: 0.19−0.43 mmol/kg-day) and 0.51 mmol/kg-day (95% CI: 0.44−0.57 mmol/kg-day), respectively. - Dosing regimen of 3 days inconsistent with OECD TG No. 414 recommendations - Teratogen screening study via i.p. injection not a relevant route of exposure - Poor documentation of administered doses and dose groups; study authors did not report the number of treatment groups or administered doses. - Teratogenic effects were observed at doses that also caused lethality in non-pregnant female mice (*i.e.,* LD_{01} and tD_{05} overlap).
Abbreviations: DMSO = dimethyl sulfoxide; GD = Gestation Day; i.p. = intraperitoneal; LOAEL = Lowest-observed-adverse-effect-level; NOAEL = No-observed-adverse-effect-level; PBS = Phosphate-buffered saline				

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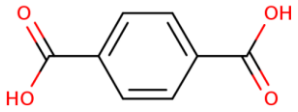
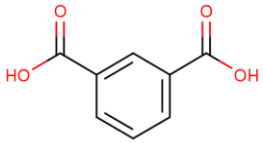
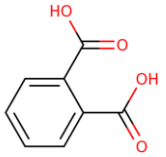
	Terephthalic Acid	Isophthalic Acid	*o*-Phthalic Acid
CASRN	100-21-0	121-91-5	88-99-3
Chemical Structure			
Urinary Bladder Calculi/stones	• ↑ Stones in 13-week dietary study of Wistar rats (NOAEL/LOAEL: None/ 2100–2500 mg/kg-d)[a] • ↑ Calculi in 15-week dietary study of albino rats (NOAEL/LOAEL: 1220–1456/ 3837–4523 mg/kg-d)[b] • ↑ Calculi in 2-year dietary study of F344 rats (NOAEL/LOAEL: 142/ 1000 mg/kg-d)[c] • ↑ Stones in 2-year dietary study of Wistar rats (NOAEL/LOAEL: 1000/ 2500 mg/kg-d)[d] • ↑ Calculi in parental and F1 offspring in one-generation studies of CD and Wistar rats (NOAEL/LOAEL: 1000/ 2500 mg/kg-d)[e]	• Not observed at doses as high as 2,500 mg/kg-day in 90-day dietary study of Wistar rats[f] (Note: Comparative study. Reference reports ↑ bladder stones at 2100–2500 mg/kg-day terephthalic acid in 90-day dietary study of Wistar rats)	• Not observed in 2-year dietary study of F344 rats at doses as high as 750 mg/kg-d[g] • Not observed in 2-year dietary study of B6C3F1 mice at doses as high as 3606–4904 mg/kg-d[g]
Urinary Bladder Pathology	• ↑ Hyperplasia in 13-week dietary study of Wistar rats (NOAEL/LOAEL: None/ 2100–2500 mg/kg-d) [a] • ↑ Hyperplasia in 15-week dietary study of albino rats (NOAEL/LOAEL: 1220–1456/ 3837–4523 mg/kg-d)[b] • ↑ Hyperplasia in 2-year dietary study of F344 rats (NOAEL/LOAEL: 142/ 1000 mg/kg-d)[c] • ↑ Hyperplasia, chronic cystitis, calculus after 30, 60, and 90 days of dietary exposure for CD and Wistar rats (NOAEL/LOAEL: 2,500/ 5000 mg/kg-d)[e]	• Not observed at doses as high as 2,500 mg/kg-day in 90-day dietary study of Wistar rats[f] (Note: Comparative study. Reference reports ↑ hyperplasia at 2100–2500 mg/kg-day terephthalic acid in 90-day dietary study of Wistar rats)	• Not observed in 2-year dietary study of F344 rats at doses as high as 750 mg/kg-d[g] • Not observed in 2-year dietary study of B6C3F1 mice at doses as high as 3606–4904 mg/kg-d[g]
Urinary Bladder Tumors	• ↑ Transitional cell adenomas in 2-year dietary study of F344 rats (NOAEL/LOAEL: 142/ 1000 mg/kg-d)[e] • ↑ Tumors in 2-year dietary study of Wistar rats (NOAEL/LOAEL: None/ 500 mg/kg-d)[d]	• No 2-year oral study available	• Not observed in 2-year dietary study of F344 rats at doses as high as 750 mg/kg-d[g] • Not observed in 2-year dietary study of B6C3F1 mice at doses as high as 3606–4904 mg/kg-d[g]

	Terephthalic Acid	Isophthalic Acid	*o*-Phthalic Acid
Urinalysis Findings	• ↑ Hematuria and proteinuria in 15-week dietary study of albino rats (NOAEL/ LOAEL: 1220–1456/ 3837–4523 mg/kg-d)[b]	• Not observed at doses as high as 2,500 mg/kg-day in 90-day dietary study of Wistar rats[f]	• Not observed in SD rats gavaged with 250 mg/kg-day *o*-phthalic acid for 14 or 28 days[h]
Renal Pathology		• ↑ Incidence of crystalluria, mild hydronephrosis, pelvic calcification in 90-day dietary study of Wistar rats (NOAEL/ LOAEL: 250/ 800 mg/kg-d)[f]	

[a] Reference: Amoco Corporation (1972) as cited by (OECD, 2001).
[b] Reference: Amoco Corporation (1970) as cited by (OECD, 2001).
[c] Reference: CIIT (1983) as cited by (OECD, 2001).
[d] Reference: Gross (1974) as cited by (OECD, 2001).
[e] Reference: CIIT (1982) as cited by (OECD, 2001).
[f] Reference: (Vogin, 1972) as cited by (OECD, 2002)
[g] Reference: (NCI, 1979)
[h] Reference: (Kwack et al., 2010; Kwack et al., 2009)

4.1.4 Evidence Integration Conclusions

This section summarizes EPA's conclusions pertaining to the systemic (Section 4.1.4.1) and developmental/reproductive effects (Section 4.1.4.2) of phthalic anhydride and *o*-phthalic acid based on EPA's integration of data from experimental animal studies, as well as mechanistic *in vitro* data. As discussed previously in Section 4.1.1, EPA identified several human epidemiological studies evaluating exposure to phthalic anhydride or *o*-phthalic acid; however, none met the inclusion and quality criteria for evidence synthesis, as detailed in Section 4.1.1. Therefore, human epidemiologic data are not further discussed in this section.

4.1.4.1 Systemic Effects

Available experimental animal studies of phthalic anhydride and *o*-phthalic acid indicate that both chemical substances have low systemic toxicity for the oral route of exposure (Table 4-2). Three studies of male Wistar rats reported no effect on any organ system following 7 to 36 days of exposure to *o*-phthalic acid at doses ranging from 850 to 5,000 mg/kg-day (Murakami et al., 1986; Oishi and Hiraga, 1980; Lake et al., 1975), while no effects were observed in male or female B6C3F1 mice exposed to up to 5,558 mg/kg-day phthalic anhydride in a 7-week dose-range finding study (NCI, 1979).

Decreases in body weight gain and/or terminal body weight have been observed consistently across most other studies of phthalic anhydride and *o*-phthalic acid, typically at the highest tested doses (Table 4-2). Dam weight gain and F1 offspring body weight on postnatal day (PND) 90 was significantly reduced following exposure to 1,763 to 2,981 mg/kg-day *o*-phthalic acid on GD 7 through GD 16 (Rahmani et al., 2015; Ema et al., 1997). Reduced terminal body weight and/or weight gain has also been observed in a 7-week dose-range finding study of male and female F344 rats at doses of 3,705 mg/kg-day phthalic anhydride (NCI, 1979); and in 2-year dietary studies of male F344 rats and male and female B6C3F1 mice fed diets containing 556 and 1,336 to 1,817 mg/kg-day phthalic anhydride, respectively (NCI, 1979). While the majority of studies report decreased body weight at higher doses, two studies in male SD rats reported decreased terminal body weight following 14 or 28 days of exposure to a single dose of 250 mg/kg-day *o*-phthalic acid via gavage (Kwack et al., 2010; Kwack et al., 2009).

Altogether, the reasonably available animal toxicology studies generally support low systemic toxicity following oral exposures to *o*-phthalic acid and phthalic anhydride. Indeed, most studies report decreases in body weight at high doses (*i.e.,* 556 mg/kg-day or higher) and do not report evidence to suggest target-organ specific toxicity. Moreover, of the eleven reasonably available studies in mice and rats, five reported no-effect levels over 1,000 mg/kg-day following gavage or drinking water exposure and LOAELs of most studies reflect decreases in body weight or food consumption ranging from 250 to 5,000 mg/kg-day. Of note, the limit dose of 1,000 mg/kg-day is typically recommended by OECD TGs for subchronic and chronic toxicity testing (OECD, 2018a, 2009).

Consistent with phthalic anhydride and *o*-phthalic acid having low systemic toxicity, neither chemical substance showed any activity across 218 *in vitro* ToxCast assays (Table 4-1). Consistent with EPA's draft conclusion, OECD (2005), Australia NICNAS (2013), and Health Canada (2019) have also concluded that phthalic anhydride has low systemic toxicity. EPA further considers body weight effects for dose-response assessment in Section 4.1.5.

4.1.4.2 Developmental and Reproductive Effects

Reasonably available information from animal toxicology studies and mechanistic evidence indicate that phthalic anhydride and *o*-phthalic acid are not directly toxic to the developing fetus. Developmental effects are not observed at doses below those that elicit maternal toxicity. In Ema et al. (1997), slight

(4%) reductions in male fetus weight (females unaffected) and a slight (7%) decrease incidence in number of ossification centers was observed at 2,981 mg/kg-day (Ema et al., 1997). However, these effects were limited to the high-dose group (2,981 mg/kg-day), were of questionable adversity, and coincided with maternal toxicity. Moreover, the LOAEL of 1,763 mg/kg-day for maternal toxicity based on a decrease in body weight gain (18–59%) and feed consumption (13–27%) was lower than that of the developmental LOAEL (2,981 mg/kg-day). Although Rahmani et al. (2015) reported a decrease in F1 body weight (14–25%) at 3 months at doses of 1,763 mg/kg-day and higher, that study did not provide results of any evaluation of maternal toxicity which limits the ability to interpret the results and the extent to which the effects are attributable to maternal toxicity.

Additional limitations in the dataset were identified, the most significant of which was the lack of studies with an exposure duration that encompasses the full window of development (*i.e.,* from conception to weaning). Indeed, exposure periods in the two available developmental studies (Rahmani et al., 2015; Ema et al., 1997) were both limited to GD 7 through GD 16, do not include the full period of skeletal formation, which continues through late gestation. The incomplete coverage of critical windows is a source of uncertainty; however, available data (*i.e.,* comparing studies of *o*-phthalic acid and *o*-phthalic acid isomers, including terephthalic acid and isophthalic acid) indicate that *o*-phthalic acid does not act as a nutrient complexing agent *in vivo*, as explained above which helps to in part address this uncertainty.

Although EPA also evaluated mechanistic targets of phthalic anhydride and *o*-phthalic acid using NTP's Integrated Chemical Environment and EPA's CompTox Chemicals Dashboard, neither chemical showed activities in any endpoint evaluated in these *in vitro* assays suggestive of potential for causing developmental toxicity (*e.g.,* no estrogen, androgen, other steroid hormone, or thyroid bioactivity). These data are consistent with the limited potential for phthalic anhydride and *o*-phthalic acid to elicit developmental toxicity.

4.1.5 Dose-Response Assessment for Oral Route of Exposure

4.1.5.1 Selection of Studies and Endpoints for Non-Cancer Toxicity Dose-Response Analysis

EPA considered non-cancer hazard endpoints from oral exposure studies of experimental animal models of phthalic anhydride and/or *o*-phthalic acid for dose-response analysis because the hazard profiles are similar; both chemical exhibit low systemic toxicity through the oral route. Endpoints considered for dose-response analysis primarily include reduced weight gain and/or reduced terminal body weights observed across 11 animal toxicology studies. These hazard endpoints were selected for dose-response analysis because EPA has the highest confidence in these hazard endpoints for estimating risk to human health. As further described below in the weight of scientific evidence conclusions (Section 4.1.6) as well as in the *Draft Environmental Media and General Population and Environmental Exposure for Phthalic Anhydride* (U.S. EPA, 2026f), EPA is utilizing a screening-level approach to estimate risks to the general population; therefore, the hazard endpoints were evaluated to derive a POD for use in the screening level general population risk assessment. Additional considerations for the hazard values used to estimate risks to the general population are discussed in Appendix H of the draft risk evaluation (U.S. EPA, 2026i).

4.1.5.2 Non-Cancer Endpoints for Acute, Intermediate, and Chronic Exposures

Table 4-5 summarizes the intermediate, subchronic, and chronic duration oral exposure studies considered for EPA's dose-response assessment. Across reasonably available studies, no health effects were observed that were relevant for setting an acute oral POD. Therefore, no acute oral POD was

derived. Seven studies provided endpoints relevant for non-cancer PODs for estimating risks for intermediate duration exposure scenarios, and four were identified for chronic duration exposure scenarios.

Of the 11 studies (9 in rats; 2 in mice), 8 administered *o*-phthalic acid or phthalic anhydride via dietary exposure. Of the 8 dietary studies, two 7-day studies of male Wistar rats reported no effect at the highest tested dose of *o*-phthalic acid, supporting unbounded NOAELs of 850 and 2,000 mg/kg-day (Murakami et al., 1986; Oishi and Hiraga, 1980). In another 7-week dose-range finding dietary study of male and female B6C3F1 mice, no adverse effects were observed, supporting an unbounded NOAEL of 5,558 mg/kg-day (NCI, 1979). These studies have limitations (*e.g.,* evaluated a limited number of outcomes in male rats (Murakami et al., 1986; Oishi and Hiraga, 1980) or had issues with test substance stability (NCI, 1979)), and lack sensitivity. Given all of these issues, these studies are not further considered.

The remaining five dietary studies consistently reported dose-related decreases in body weight at doses of approximately 556 mg/kg-day or higher. Two of these publications were developmental studies that reported effects on body weight following gavage exposures from GD 7 to 16 (Rahmani et al., 2015; Ema et al., 1997). Ema et al. exposed pregnant Wistar-Kyoto rats via feed with diets containing 0, 1,021, 1,763, or 2,981 mg/kg-day *o*-phthalic acid from GD 7 through GD 16. In the dams, decreased body weight gain (18–59%) and decreased food consumption were observed at doses of 1,763 mg/kg-day and higher, supporting a maternal NOAEL of 1,021 mg/kg-day, while a slight (4%) reduction in fetal male (but not female) weight was observed at 2,981 mg/kg-day, supporting a developmental NOAEL of 1,763 mg/kg-day. In a similar experiment, Rahmani et al. (2015) exposed pregnant Wistar-Kyoto rats via feed with diets containing 0, 1,763, or 2,981 mg/kg-day *o*-phthalic acid from GD 7 through GD 16. Decreased F1 offspring body weight (14–25%) was observed on PND90 in the 1,763 mg/kg-day dose group. Increases in systolic blood pressure, decreased absolute heart weight, and increased wall thickness, cross-sectional area, and wall thickness/inner diameter ratio were also observed at 1,763 mg/kg-day. This study supports a LOAEL (no NOAEL identified) of 1,763 mg/kg-day.

The NCI (1979) studies (subchronic and chronic studies in mice and rats) provided a range of candidate PODs. In the subchronic rat study, NCI (1979) reported decreased body weight (24–26%) in male and female F344 rats given 3,705 mg/kg-day phthalic anhydride in feed after 7 weeks of exposure, supporting a NOAEL of 1,853 mg/kg-day. NCI (1979) also reported a 2-year rat study, where male and female F344 rats were fed diets with 0, 278 and 556 mg/kg-day for 105 weeks. Decreased (≈10%) body weight gain was observed in high-dose male (but not female) rats compared to controls starting at week 13 and lasting until the end of study, supporting a NOAEL of 278 mg/kg-day. In the 2-year mouse study, NCI (1979) reported decreased terminal body weight (12–27%) in male and female B6C3F1 mice fed diets with 1,336 (females) to 1,817 (males) mg/kg-day phthalic anhydride (lowest dose tested) for 2 years. Notably, body weight effects coincided with increased incidences of histopathology in lung and kidney (both sexes) and adrenal cortex and thalamus (males only), based on a statistical re-analysis by U.S. EPA (1988). Overall, the chronic mouse study supports a LOAEL of 1,336 mg/kg-day based on decreased body weight gain and histopathology, with no NOAEL identified. The subchronic studies in rats and mice, as well as the chronic study in mice were not considered further for dose-response due to lack of sensitivity, and lack of observed lung, kidney, and adrenal cortex effects in other studies.

Of note, the doses reported herein reflect EPAs estimated received daily doses. Estimates were calculated using assumptions on feed consumption rate (*i.e.,* assumption of 5% body weight per day) and test substance loss (*i.e.,* to account for author reported issues with test substance stability indicating that estimated received doses may be underestimated by 18 to 26% [see discussion in Section 4.1.3]). Moreover, NCI (1979) reported a 2.59% loss of phthalic anhydride from the feed mix per day over a 14-

day period when stored at room temperature, and also reported that fresh food was prepared every 7 to 10 days. Assuming fresh diet was prepared every 10 days with a 2.59% loss per day, EPA estimated the total loss after 10 days to be 25.9%. For example, after accounting for test substance loss in the in the 2-year rat study by NCI (1979), EPA estimated the adjusted received doses of phthalic anhydride (using the adjusted values of 375 and 750 mg/kg-day) to be 278 and 556 mg/kg-day, supporting an adjusted NOAEL of 278 mg/kg-day (LOAEL of 556 mg/kg-day). Strengths of this study include that it was of a chronic duration, was well powered, and evaluated effects of phthalic anhydride in two species and both sexes.

The three gavage studies (all in rats) (Kwack et al., 2010; Kwack et al., 2009; Lake et al., 1975) provided candidate POD values ranging from 250 mg/kg-day (LOAELs) to 850 mg/kg-day (NOAEL). Lake et al. (1975) exposed male Wistar rats to 0 or 850 mg/kg-day *o*-phthalic acid for 7 days and did not note any significant findings, supporting an unbounded NOAEL of 850 mg/kg-day. However, there were several limitations of the Lake and colleagues study, including its limited scope (*i.e.,* authors focused on liver endpoints, did not evaluate body weight), only a single dose level was tested, only male rats were evaluated, and the shorter duration of exposure (*i.e.,* 7-days) compared to that of the Kwack et al. studies, which measured more outcomes and had longer durations of exposure.

Kwack et al. (2010) exposed male SD rats to 0 or 250 mg/kg-day *o*-phthalic acid for 14 days via gavage and reported decreased mean terminal body weight (14%). In a second study, the same authors (Kwack et al., 2009) exposed male SD rats to 0 or 250 mg/kg-day *o*-phthalic acid for 28 days via gavage and reported decreased mean terminal body weight (22%). Both studies support a LOAEL of 250 mg/kg-day based on decreases in terminal body weight in rats following 14 to 28 days of exposure to *o*-phthalic acid. The Kwack et al. studies contained similar limitations to the Lake study, including the fact that only a single dose level was tested, only male rats were evaluated, and only 6 rats were included in the control and dose groups.

EPA has preliminarily selected a NOAEL of 278 mg/kg-day based on an approximate 10% decrease in body weight gain at the LOAEL of 556 mg/kg-day in male F344 rats fed diets containing phthalic anhydride for 2 years (NCI, 1979). As discussed above, this NOAEL reflects the received dose of phthalic anhydride adjusted for an approximate 26% loss in test substance. The NOAEL was extrapolated to a human equivalent dose (HED) of 66 mg/kg-day using allometric bodyweight scaling to the three-quarters power (U.S. EPA, 2011b). A total uncertainty factor (UF) of 30× was selected for use as the benchmark margin of exposure (MOE) (intraspecies UF [UF_H] = 10×; interspecies UF [UF_A] = 3×). Consistent with EPA guidance (2022, 2002, 1993), EPA reduced the UF_A from a value of 10× to 3× because allometric body weight scaling was used to adjust the POD to obtain an HED.

EPA selected the NOAEL of 278 mg/kg-day from the chronic dietary rat study by NCI (1979) to serve as the basis of the oral POD over the LOAEL of 250 mg/kg-day from the intermediate duration oral gavage studies by Kwack et al. (2010; 2009) due to several limitations and uncertainties associated with the studies by Kwack et al. that reduce EPA's confidence in using the study quantitatively in risk characterization. Primary limitations of the studies conducted by Kwack et al. include the fact that only a single dose level was evaluated, study authors evaluated male (but not female) rats, and the sample size was small (5–6 rats per dose group). Additionally, Kwack et al. exposed rats via oral gavage, which is expected to lead to high serum concentrations and observed body weight effects may be related to the maximum (or peak) serum concentration (*i.e.,* C_{max}) of *o*-phthalic acid. In contrast, the body weight effects observed in the dietary study by NCI (1979) are likely related to the total amount of phthalic acid in systemic circulation (*i.e.,* area under the blood concentration-time curve or AUC). EPA considers the

likely toxicokinetics associated with the NCI dietary study to be more reflective of potential general population exposures to phthalic acid through TSCA releases.

Table 4-5. Summary of Candidate PODs from Oral Exposure Studies

Target Organ/ System	Relevant Duration(s)	Test Substance	Brief Study Details (Species (sex), Duration; Exposure Route/ Method; Doses [mg/kg-day]) (Reference)	Study POD/Type (mg/kg-day)	Effect at LOAEL	HED (mg/kg-day)	HEC (mg/m^3) [ppm]	Ufs [a b c]	Study Quality[d]
Studies of phthalic anhydride									
Nutritional/ Metabolic	Chronic	Phthalic anhydride	F344 Rats (both sexes); 2 years; Oral/ dietary; 0, 375, 750 (doses adjusted for test substance loss: 0, 278, 556) (NCI, 1979)	NOAEL = 278	↓ Body weight gain (males only)	66	358 [52.6]	$UF_A = 3$ $UF_H = 10$ Total UF = 30	Medium
Nutritional/ Metabolic; Respiratory; Renal; Adrenal; Thalamus	Chronic	Phthalic anhydride	B6C3F1 mice (both sexes); 2 years; Oral/ dietary; 0, 1,817, 3,634 (males); 0, 1,336, 2,672 (females) (NCI, 1979)	LOAEL = 1,803	↓ (12–27%) terminal body weight (both sexes); ↑ incidence of histopathology in lung & kidney (both sexes) and adrenal cortex and thalamus (males only)	240	1,305 [192]	$UF_A = 3$ $UF_H = 10$ $UF_L = 10$ Total UF = 300	Medium
Nutritional/ Metabolic	Subchronic	Phthalic anhydride	F344 Rats (both sexes); 7 weeks; Oral/ dietary; 0, 230, 463, 926, 1,853 (NCI, 1979)	NOAEL = 926	↓ (24–26%) Terminal body (both sexes)	591	3,217 [473]	$UF_A = 3$ $UF_H = 10$ Total UF = 30	Medium
N/A	Subchronic	Phthalic anhydride	B6C3F1 mice (both sexes); 7 weeks; Oral/ dietary; 0, 689, 1,389, 2,779, 5,558 (NCI, 1979)	NOAEL = 5,558	None (no effect study)	997	5,427 [799]	$UF_A = 3$ $UF_H = 10$ Total UF = 30	Medium
Studies of *o*-phthalic acid									
Nutritional/ Metabolic	Intermediate	*o*-Phthalic acid	SD Rats (males only); 14 days; Oral/ gavage; 0, 250 (Kwack et al., 2010)	LOAEL = 250	↓ (14%) Terminal body	59	322 [47]	$UF_A = 3$ $UF_H = 10$ $UF_L = 10$ Total UF = 300	High
Nutritional/ Metabolic	Intermediate	*o*-Phthalic acid	SD Rats (males only); 28 days; Oral/ gavage; 0, 250 (Kwack et al., 2009)	LOAEL = 250	↓ (22%) Terminal body	59	322 [47]	$UF_A = 3$ $UF_H = 10$ $UF_L = 10$	High

Target Organ/ System	Relevant Duration(s)	Test Substance	Brief Study Details (Species (sex), Duration; Exposure Route/ Method; Doses [mg/kg-day]) (Reference)	Study POD/Type (mg/kg-day)	Effect at LOAEL	HED (mg/kg-day)	HEC (mg/m^3) [ppm]	Ufs [a b c]	Study Quality[d]
								Total UF = 300	
N/A	Intermediate	*o*-Phthalic acid	Wistar Rats (males only); 7 days; Oral/ gavage; 0, 850 (Lake et al., 1975)	NOAEL = 850	None (no effect study)	201	1,094 [161]	UF_A = 3 UF_H = 10 Total UF = 30	Medium
Nutritional/ Metabolic	Intermediate	*o*-Phthalic acid	Pregnant Wistar Rats (females); GD 7–16; Oral/ dietary; 0, 1,021, 1,763, 2,981 (Ema et al., 1997)	NOAEL = 1,021	↓ maternal weight gain and food consumption	241	1,314 [193]	UF_A = 3 UF_H = 10 Total UF = 30	High
Nutritional/ Metabolic; Cardiovascular	Intermediate	*o*-Phthalic acid	F1 Wistar Rats (both sexes); GD 7–16; Oral/ dietary; 0, 1,763, 2,981 (Rahmani et al., 2015)	LOAEL = 1,763	↓ F1 offspring body weight on PND 90, cardiovascular effects	417	2,268 [334]	UF_A = 3 UF_H = 10 UF_L = 10 Total UF = 300	Medium
N/A	Intermediate	*o*-Phthalic acid	Wistar Rats (males only); 7 days; Oral/ dietary; 0, 2,000 (Oishi and Hiraga, 1980)	NOAEL = 2,000	None (no effect study)	473	2,573 [379]	UF_A = 3 UF_H = 10 Total UF = 30	– [e]
N/A	Intermediate	*o*-Phthalic acid	Wistar Rats (males only); 34–36 days; Oral/ dietary; 0, 500, 5,000 (Murakami et al., 1986)	NOAEL = 5,000	None (no effect study)	1,182	6,434 [947]	UF_A = 3 UF_H = 10 Total UF = 30	Medium

↓ = statistically significant decrease; ↑ = statistically significant increase; F1 = first-generation; GD = Gestation Day; HEC = human equivalent concentration; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect-level; ND = no data; POD = point of departure; UF = uncertainty factor; UF_A = Interspecies UF; UF_H = Intraspecies UF; UF_L = LOAEL-to-NOAEL UF

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011b), the UF_A was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics.

[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to *o*-phthalic acid/phthalic anhydride.

[c] EPA used a UF_L of 10 to account for the uncertainty inherent in extrapolating from the LOAEL-to-NOAEL.

[d] Overall data quality determinations were made for these studies unless otherwise stated and further details are provided in the *Draft Systematic Review Protocol for Phthalic Anhydride* (U.S. EPA, 2026j).

[e] Study quality evaluation was not conducted for reference.

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4.1.6 Weight of Scientific Evidence Conclusions: Oral POD

EPA reviewed reasonably available evidence of oral exposure to phthalic anhydride and o-phthalic acid, which have similar hazard profiles (*i.e.,* low systemic toxicity through the oral route). EPA has preliminarily selected an HED of 66 mg/kg-day (NOAEL = 278 mg/kg-day), which is based on decreased in body weight gain in male F344 rats fed diets containing phthalic anhydride for 2 years (NCI, 1979). A total UF of 30× was selected for use as the benchmark MOE (based on a UF_A of 3× and a UF_H of 10×) to characterize risks in the screening-level general population assessment.

The draft POD selected for use in the screening-level general population assessment is supported by the following weight of scientific evidence considerations:

- Eleven reasonably available animal toxicology studies in mice and rats demonstrate the low systemic toxicity of phthalic anhydride and/or *o*-phthalic acid via the oral route. No consistent evidence of any specific target organ toxicity was observed, and the only consistent effect observed across studies was reduced body weight gain and/or terminal body weight. Consistently, existing assessments by OECD (2005), Australia NICNAS (2013), and Health Canada (2019) have also concluded that phthalic anhydride has low systemic toxicity via the oral exposure route.
- The proposed POD is supported by consistency with mechanistic evidence from *in vitro* assays, which indicate a lack of bioactivity of phthalic anhydride and *o*-phthalic acid across all tested assays and further support the conclusion that *o*-phthalic acid has low potential for systemic toxicity via the oral route.
- The proposed POD is health protective and is lower than the majority of candidate PODs (*i.e.,* 8) considered by EPA, which reflected NOAEL's based on no effect or similarly based on decreases in body weight.
- Although more sensitive PODs were available from the two short-term gavage studies of o-phthalic acid by Kwack et al. (Kwack et al., 2010; Kwack et al., 2009), the proposed POD from the NCI study (1979) is derived from a dietary study of phthalic anhydride. EPA preferred the proposed POD from the NCI study because of the limitations associated with the Kwack et al. studies, including the small number of animals, and less relevant gavage route of exposure compared to dietary. EPA considers the expected toxicokinetics associated with the NCI dietary study to be more reflective of potential general population exposures to phthalic acid through TSCA releases. In addition to this reason, EPA conducted a sensitivity analysis using the more conservative candidate POD (see Appendix H of the Draft Risk Evaluation (U.S. EPA, 2026i)).
- Based on reasonably available data, the proposed POD is expected to be protective of sensitive lifestages. The developmental study by Ema et al. (1997) reported information that can be used to evaluate maternal toxicity; the lowest doses where maternal toxicity is observed (*i.e.,* 1,763 mg/kg-day) are approximately an order of magnitude greater than the proposed PODs (*i.e.,* 250 to 278 mg/kg-day), indicating the proposed POD is protective is sensitive life stages. Moreover, studies do not indicate developmental toxicity at doses below those which elicit maternal toxicity (*i.e.,* 1,763 mg/kg-day).
- Uncertainties remain in the evidence base, including the lack of reasonable available epidemiological studies evaluating phthalic anhydride or *o*-phthalic acid, and limited number of developmental and reproductive toxicity studies. The two available reproductive and developmental studies (Rahmani et al., 2015; Ema et al., 1997) did not expose animals during the complete period of gestational development, which is a source of uncertainty. However, EPA

reviewed available data on *o*-phthalic acid and *o*-phthalic acid isomers, including terephthalic acid and isophthalic acid, which indicate that *o*-phthalic acid does not act as a nutrient complexing agent *in vivo*, which may address this uncertainty to some extent.

- There is also uncertainty because of the wide range of effect levels at which body weight effects are observed, where LOAEL's of most of the 11 studies reflect decreases in body weight or food consumption ranging from 250 to 5,000 mg/kg-day.

4.2 Dermal Exposure

Reasonably available information pertaining to the human health effects of dermal exposure to phthalic anhydride or its immediate hydrolysis product, *o*-phthalic acid, are provided in Section 4.2.1 and 4.2.2, respectively.

4.2.1 Phthalic Anhydride

Both human and animal data were identified that provide information on the effects of dermal exposure to phthalic anhydride. All of the reasonably available information identified by EPA for the dermal route evaluated skin sensitization and related immune system effects.

In the EU, phthalic anhydride is classified (GHS) as Skin Sens. 1 (H317: May cause an allergic skin reaction) (https://chem.echa.europa.eu/100.001.461/overview?searchText=85-44-9, accessed February 2, 2026). Additionally, OECD (2005), Health Canada (2019), Australia NICNAS (2013), and ACGIH (2025) have also identified phthalic anhydride as a skin sensitizer. In contrast, *o*-phthalic acid is not a skin sensitizer (see Section 4.2.2). Skin sensitization is an immunological process that occurs in two phases: the induction of sensitization and the subsequent elicitation of the immune reaction (OECD, 2014). EPA developed a detailed hazard characterization and AOP analysis for skin sensitization. Evidence from humans, experimental animal models, as well as *in chemico* and *in vitro* test methods, are described in Section 4.2.1.1. Available data in Section 4.2.1.1 is organized by "Key Event" (KE) in the OECD (2014) AOP for skin sensitization. EPA's weight of scientific evidence conclusions and dose-response assessment for dermal sensitization presented in Sections 4.2.1.3 and 4.2.1.4, respectively. Skin sensitization is most relevant to acute exposures, as a single exposure to phthalic anhydride might elicit immunological events during the induction phase of skin sensitization.

Phthalic anhydride is also considered a skin irritant based on guideline (OECD TG 404) and non-guideline studies in rabbits and observations in humans reported by OECD (2005), Australia NICNAS (2013), and ACGIH (2025). In the EU, phthalic anhydride is classified (GHS) as Skin Irrit. 2 (H315: Causes skin irritation). However, original copies of skin irritation studies were not reasonably available to EPA for independent review because the studies cited by OECD (2005) were in German and/or cited as unpublished study reports. The same studies are referenced by the European Chemicals Agency (ECHA). Therefore, skin irritation results as reported by ECHA are summarized below but are not discussed further in this draft assessment:

> In 2 valid skin irritation studies, phthalic anhydride was found slightly/moderately irritating (Thyssen, Muermann). In 2 reliable published studies the test substance was characterised as not irritating or slightly irritating. In a valid eye irritation study with phthalic anhydride conjunctivae effects were not reversible within 7 days (observation period) (Thyssen). Published studies found phthalic anhydride irritating/extremely irritating.

No reasonably available studies investigating other systemic endpoints following dermal exposure to phthalic anhydride were identified by EPA.

4.2.1.1 AOP for Skin Sensitization – Summary of *In Chemico*, *In Vitro*, and *In Vivo* Data

OECD has published an AOP for skin sensitization initiated by covalent binding to proteins. Furthermore, *in chemico*, *in vitro*, and *in vivo* test guidelines are available to evaluate the molecular initiating event (MIE), KEs, and the adverse outcome in the AOP (OECD, 2014). The AOP and available test guidelines form the basis of EPA's *2018 Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing* (EPA-HQ-OPP-2016-0093-0090) and the subsequent OECD Defined Approaches (DAs) on skin sensitization (OECD, 2025b).

This section summarizes available *in chemico, in vitro*, and *in vivo* data for the skin sensitizing potential of phthalic anhydride. Available data are organized by KE in the OECD (2014) AOP for skin sensitization, which are shown in Figure 4-1 and summarized briefly below. KE 1 through KE 4 correspond to the induction phase of sensitization, while the adverse outcome, inflammation upon challenge with allergen, corresponds to elicitation of the immune reaction.

- **KE 1 – Covalent binding to proteins (OECD TG 442C):** KE 1, the MIE, involves covalent binding of an electrophilic chemical to cysteine or lysine residues in epidermal proteins. Covalent binding forms a hapten complex, which can be recognized by immune cells and trigger subsequent KEs in the AOP leading to skin sensitization.
- **KE 2 – Events in keratinocytes (OECD TG 442 D):** In keratinocytes, the hapten complex can react with cell surface proteins and/or endogenous proteins and activate response pathways, including the release of pro-inflammatory cytokines (*e.g.,* interleukin-18 [IL-18]) and induction of cytoprotective cellular pathways (*e.g.,* the Nrf2-Keap1-ARE regulatory pathway [nuclear factor erythroid 2-related factor 2 (Nrf2), Kelch-like ECH-associated protein 1 (Keap1), antioxidant response element (ARE)]). Induction of the Nrf2-Keap1-ARE regulatory pathway involves oxidation or covalent modification of Keap1, which is the negative regulator of the Nrf2 transcription factor. When the reactive cysteines on Keap1 are oxidized or covalently modified, Nrf2 is free to traverse to the nucleus to activate genes that contain an ARE (antioxidant response element) in the promoter sequence, leading to induction of pro-inflammatory cytokines and cyto-protective cellular pathways.
- **KE 3 – Events in dendritic cells (DCs) (OECD TG 442 E):** In parallel with KE 2, additional epidermal responses include activation of immature epidermal DCs, known as Langerhans cells. Immature DCs can internalize the hapten complex formed as part of KE 1, and then maturing cells migrate from the epidermis to the dermis, and then to the proximal lymph nodes. In the lymph nodes, mature DCs display the hapten-protein complex to naïve T-cells via a major histocompatibility complex (MHC). Activation of DCs also leads to increased chemokine and cytokine secretion, as well as changes in expression of chemokine receptors.
- **KE 4 – T-cell proliferation (OECD TG 429):** Activated DCs (KE 3) migrate from epidermis to proximal lymph nodes where they can present a hapten-protein complex via an MHC to naïve T-cells. If presented with a foreign peptide, T-cells are activated to form a memory T-cell, which subsequently proliferate.
- **Adverse Outcome – Inflammation upon challenge with allergen:** The adverse outcome is an inflammatory response upon challenge with an allergen. Memory T-cells reactivated by hapten presentation by activated mature DCs will induce inflammation and allergic contact dermatitis in humans or its rodent equivalent, contact hypersensitivity.

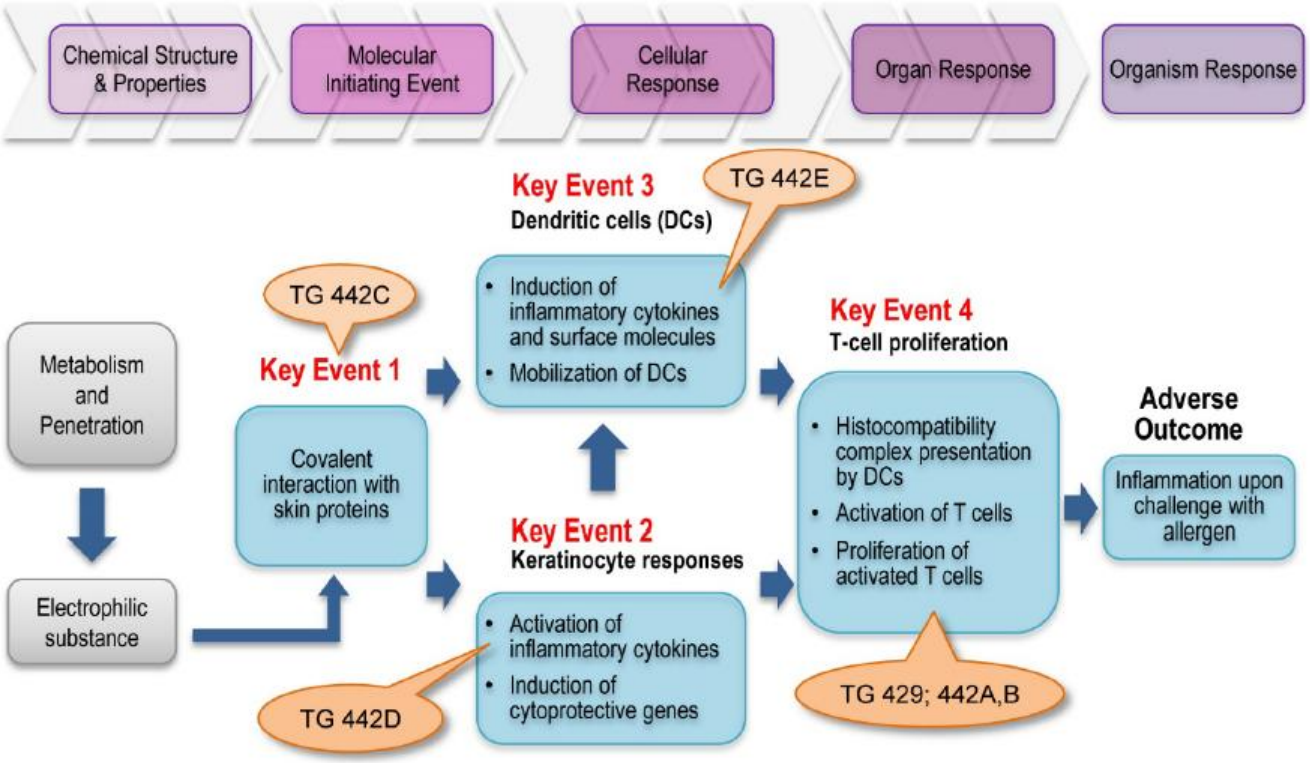

Figure 4-1. AOP for Skin Sensitization Initiated by Covalent Binding to Proteins
Adapted from EPA-HQ-OPP-2016-0093-0090.

4.2.1.1.1 KE 1: Covalent Interaction with Skin Proteins

KE 1 (covalent binding to skin proteins) can be evaluated *in chemico* using the Direct Peptide Reactivity Assay (DPRA), the Amino Acid Derivative Reactivity Assay (ADRA), or the kinetic DPRA (kDPRA). These *in chemico* assays are described in OECD TG No. 442C (In Chemico Skin Sensitization) (OECD, 2023a). Briefly, the DPRA evaluates depletion of synthetic peptides containing lysine or cysteine, while the ADRA evaluates depletion of synthetic amino acid derivatives containing lysine or cysteine as markers of chemical reactivity. The kDPRA assay measures the reactivity of chemicals towards synthetic peptides containing cysteine in a time- and concentration-dependent manner.

Phthalic anhydride has been evaluated twice in the DPRA and once in the kDPRA (Table 4-6). Although conducted prior to the establishment of OECD TG No. 442C (OECD, 2023a), available studies were conducted in a manner consistent with OECD TGs. Bauch et al. (2012) was considered *Acceptable* for qualitative use as part of the weight of scientific evidence (*i.e.,* in Section 4.2.1.3) primarily due to use of low purity test substance (reported to be 5%), and the fact that the ratios of test substance to peptide varied significantly from OECD TG 442C (*i.e.,* OECD TG 442C requires ratios of 1:10 [Cys] and 1:50 [Lys], while ratios used in the study were 1:15 [Cys] and 1:3 [Lys]). However, Bauch et al. (2012) was considered appropriate for use qualitatively as part of the weight of scientific evidence.

The remaining peer-reviewed publications reporting KE 1 data for phthalic anhydride, which were considered *Acceptable* for quantitative use, tested large (*i.e.,* 38–145) groups of chemicals as part of validation studies that are cited in OECD TG No. 442C in support of the described test guidelines (Wareing et al., 2017; Gerberick et al., 2004). Phthalic anhydride gave a positive result in the DPRA assay with mean peptide depletion value of 38% (Gerberick et al., 2004), which indicates that phthalic anhydride has moderate peptide reactivity under OECD TG No. 442C. Similarly, a positive result was

obtained in the kDPRA with a log k_{max} value of −0.67 s^{-1} M^{-1}. Under OECD TG No. 442C, the obtained log k_{max} value supports a UN GHS subcategory 1A classification. Table 4-6 summarizes studies deemed acceptable for use quantitatively in dose-response analysis (Section 4.2.1.3).

In addition, phthalic anhydride has been demonstrated to covalently bind to human hemoglobin *in vitro*. Jeppsson et al. (2008) identified six covalent bindings sites for phthalic anhydride on human hemoglobin, including adducts on the N-terminal valine in both the α- and β-chains, as well as on four lysine animo acids (*i.e.,* lysine 16 and 61 on the α-chain; lysine 66 and 144 on the β-chain).

The mechanism by which phthalic anhydride covalently interacts with proteins has been proposed to be through protein acylation, which is a reaction in which the electrophilic acyl group preferentially reacts with nucleophiles, such as lysine and cysteine residues (Johnson et al., 2022; Wareing et al., 2017; Piroird et al., 2015; Bauch et al., 2012; Enoch et al., 2009). A recent *in chemico* analysis with a large set of sensitizers demonstrated that acid anhydrides as a class, including phthalic anhydride, preferentially bind lysine residues via acylation (Krutz et al., 2021; Dik et al., 2016). This mechanism is supported by DPRA assay results, in which phthalic anhydride preferentially depleted synthetic lysine peptides (Table 4-6).

Table 4-6. Summary of Phthalic Anhydride Data for KE 1 in Skin Sensitization AOP

Assay	Result	Remarks	Reference
DPRA	Positive	- Peptide depletion: lysine (31.3%), cysteine (16.7%), mean (24%) - Reactivity class: Moderate	(Bauch et al., 2012)
DPRA	Positive	- Peptide depletion: lysine (75%), cysteine (1.9%); mean (38%) - Reactivity class: Moderate	(Natsch et al., 2013) [a]
kDPRA	Positive	- Log K_{max} = −0.67 s^{-1} M^{-1} - Log K_{max} value supports UN GHS subcategory 1A	(Wareing et al., 2017)

DPRA = direct peptide reactivity assay; kDPRA = kinetic DPRA; k_{max} = maximum rate constant (in $S^{-1}M^{-1}$) determined from the reaction kinetics in the kDPRA; UN GHS = United Nations Globally Harmonized System of Classification and Labeling of Chemicals
[a] Natsch et al. (2013) references DPRA data originally from (Gerberick et al., 2004).
Grey highlighting indicates studies considered *Acceptable* for use in quantitative dose-response in Section 4.2.1.3.

4.2.1.1.2 KE 2: Keratinocyte Cellular Response

KE 2 (keratinocyte activation) can be assessed *in vitro* using the ARE-Nrf2 luciferase KeratinoSens test method, the ARE-Nrf2 luciferase LuSens test method, or the Epidermal Sensitisation Assay (EpiSensA). These *in vitro* assays are described in OECD TG No. 442D (In Vitro Skin Sensitization) (OECD, 2024). The KeratinoSens and LuSens test methods evaluate keratinocyte activation using a luciferase reporter under the control of the ARE, which is activated by Nrf2, while the Epidermal Assay involves gene expression analysis of marker genes associated with the inflammatory response of keratinocyte activation (*e.g., ATF3*, *IL-8*) as well as the induction of cytoprotective gene pathways.

Phthalic anhydride has been evaluated twice in the KeratinoSens assay (Natsch et al., 2013; Bauch et al., 2012) and once in the LuSens assay (Bauch et al., 2012). Available studies were conducted prior to the establishment of OECD TG No. 442D, however, available assays were generally conducted in a manner consistent with OECD TGs. Further, the peer-reviewed publications reporting KE 2 data for phthalic anhydride tested large (*i.e.,* 54–145) groups of chemicals as part of validation studies that are cited in OECD TG No. 442D in support of the described test guidelines (Natsch et al., 2013; Bauch et al., 2012). Only the KeratinoSens assay from Natsch et al. (2013) was considered *Acceptable* for quantitative use. Across available studies, phthalic anhydride did not induce luciferase activity above the threshold of

50% in any assay at concentrations as high as 2,000 µM and was therefore considered negative under the conditions of the studies (Table 4-7). However, Bauch et al. (2012), did not have sufficient data for this KE to use quantitatively in for dose-response analysis (Section 4.2.1.3). Moreover, the study did not report information to determine the IC_{50} (the concentration for which 50% reduction in cellular viability occurs) for the KeratinoSens assay, and did not report CV75 (the concentration at which 75% cell viability occurs) for the LuSens assay. Therefore, these studies (Bauch et al., 2012) were used qualitatively as part of the weight of scientific evidence.

However, KeratinoSens and LuSens assay results need to be interpreted with caution due to the chemistry and physical and chemical properties expected of phthalic anhydride in the context of these assays. As discussed in OECD TG No. 442D, chemicals that show exclusive reactivity towards lysine-residues may show negative results in both the KeratinoSens and LuSens assays (OECD, 2024). This is because the mechanism leading to activation of the Keap1-Nrf2-ARE pathway in both assays involves the interaction of electrophilic chemicals with nucleophilic thiols in cysteine residues of Keap-1. As discussed above in Section 4.2.1.1.1, phthalic anhydride covalently interacts with proteins through transfer of acyl groups, and results from two DPRA assays indicate the phthalic anhydride preferentially reacts with lysine residues over cysteine residues. Another consideration is that phthalic anhydride rapidly hydrolyzes to *o*-phthalic acid (a non-sensitizer) in aqueous solutions, with hydrolysis half-lives ranging from approximately 30 to 90 seconds, depending upon temperature (U.S. EPA, 2026h). Rapid hydrolysis of phthalic anhydride to *o*-phthalic acid in aqueous cell culture medium is therefore expected to occur in the KeratinoSens and LuSens assays, which likely contributes to the negative results in these assays. EpiSensA is a more appropriate model for evaluating KE 2 for this chemical; because the assay involves an air-liquid interface where chemicals are applied directly to the surface of the reconstituted human epidermis model, rather than in an aqueous medium. However, phthalic anhydride has not yet been tested in EpiSensA. Table 4-7 summarizes studies deemed acceptable for use quantitatively in dose response analysis (Section 4.2.1.3) or qualitatively.

Table 4-7. Summary of Phthalic Anhydride Data for KE 2 in Skin Sensitization AOP

Assay	Result	Remarks	Reference
KeratinoSens	Negative[a]	- $EC_{1.5}$ and IC_{50} values >2,000 µM[a b c] (highest concentration tested)	(Natsch et al., 2013)
KeratinoSens	Negative[a]	- $EC_{1.5}$ value >2,000 µM[b] (highest concentration tested) - IC_{50} value not reported[c]	(Bauch et al., 2012)
LuSens	Negative[a]	- $EC_{1.5}$ value >2,000 µM[b] (highest concentration tested) - CV75 value not reported[d]	(Bauch et al., 2012)

[a] Note that rapid hydrolysis of phthalic anhydride to *o*-phthalic acid in aqueous cell culture medium is expected to occur, which may lead to false negative results in the KeratinoSens and LuSens assays.
[b] $EC_{1.5}$ is the value representing the concentration for which luciferase activity is induced above the 1.5-fold threshold (*i.e.,* 50% increase in luciferase activity) (OECD, 2024).
[c] IC_{50} is the concentration for which 50% reduction in cellular viability occurs (OECD, 2024).
[d] CV_{75} is the concentration at which 75% cell viability occurs (OECD, 2024).
Grey highlighting indicates studies considered *Acceptable* for use in quantitative dose response in Section 4.2.1.3. Studies not highlighted in gray did not report sufficient information for use in quantitative dose-response and were used qualitatively as part of the weight of scientific evidence.

4.2.1.1.3 KE 3: Dendritic Cell Response

KE 3 (DC activation) can be evaluated by the Human Cell Line Activation test (h-CLAT), U937 cell line activation test (U-SENS), Interleukin-8 Reporter Gene Assay (IL-8 Luc assay), or Genomic Allergen Rapid Detection for Assessment of skin sensitizers assay (GARD skin). These *in vitro* assays

are described in OECD TG No. 442E (In Vitro Skin Sensitization) (OECD, 2023b). These assays evaluate DC activation by quantifying cell surface marker expression of CD86 and/or CD54 (h-CLAT and U-SENS); IL-8 and GAPDH activity using a luciferase reporter (IL-8 Luc assay); or gene expression changes (GARD skin).

Phthalic anhydride had been evaluated in three U-SENS assays (Piroird et al., 2015; Natsch et al., 2013; Bauch et al., 2012), two h-CLAT assays (Bauch et al., 2012; Nukada et al., 2012), and one GARDskin assay (Table 4-8). Available U-SENS and h-CLAT studies were conducted prior to the establishment of OECD TG No. 442E, however, available assays were generally conducted in a manner consistent with OECD TGs. Further, the peer-reviewed publications reporting KE 2 data for phthalic anhydride tested large (*i.e.,* 54 to 175) groups of chemicals as part of validation studies that are cited in OECD TG No. 442E in support of the described test guidelines (Piroird et al., 2015; Natsch et al., 2013; Bauch et al., 2012; Nukada et al., 2012). The only assay considered *Acceptable* for quantitative use was the h-CLAT by Nukada et al. (2012). The available U-SENS and one of the available h-CLAT studies (Bauch et al., 2012) did not have sufficient data for this KE to use quantitatively. Moreover, the three available U-SENS assays as well as the h-CLAT assay did not report all requisite data *(i.e.,* missing CV70, CV75, or CD86 information) for use in quantitative dose-response in Section 4.2.1.3. Therefore, these studies were used qualitatively as part of the weight of scientific evidence.

Phthalic anhydride was considered negative in both h-CLAT assays (Bauch et al., 2012; Nukada et al., 2012) and in two of three U-SENS assays (Piroird et al., 2015; Bauch et al., 2012) (Table 4-8). In the third U-SENS assay conducted by Natsch et al. (2013), phthalic anhydride induced a stimulation index (SI) for CD86 expression above the threshold of 150%, supporting an EC_{150} value (effective concentration showing the relative fluorescent intensity values of 150%) of 1,080 µM phthalic anhydride, indicating a positive result, albeit a weaker response given the relatively high-test concentration.

EPA collaborated with NICEATM and Inotiv to nominate phthalic anhydride for testing by NICEATM in the GARDskin assay. The nomination was accepted, and NICEATM initiated testing on the potential for phthalic anhydride to cause skin sensitization using the GARDskin assay (NTP, 2026). Under the conditions of the study, phthalic anhydride gave a positive result for sensitization (Table 4-8).

U-SENS and h-CLAT assay results need to be interpreted with caution for phthalic anhydride. As discussed above in Section 4.2.1.1.2, phthalic anhydride rapidly hydrolyzes to *o*-phthalic acid (a non-sensitizer) in aqueous solutions, with hydrolysis half-lives ranging from approximately 30 to 90 seconds, depending upon temperature (U.S. EPA, 2026h). Rapid hydrolysis of phthalic anhydride to *o*-phthalic acid in aqueous cell culture medium likely leads to false negative results in these assays. Indeed, Piroird et al. (2015) speculate that rapid hydrolysis of phthalic anhydride to *o*-phthalic acid in cell culture medium may account for the negative result obtained in the U-SENS assay. Additionally, in a follow-up publication to the h-CLAT study by Nukada et al. by the same research group, Takenouchi et al. (2013) note that although phthalic anhydride has a Log K_{OW} less than 3.5,[4] phthalic anhydride was "hard to dissolve in both DMSO and culture medium, and the maximal achievable dose was limited. Thus, the testing condition might be insufficient for this chemical to induce a positive response [in the h-CLAT assay]." A series of studies by Narita et al. demonstrated that false-negatives in h-CLAT are due to phthalic anhydride hydrolysis in an aqueous medium (Narita et al., 2017) and that this can be avoided by testing phthalic anhydride in a "modified h-CLAT," which applies a shorter-duration of exposure in

[4] Per OECD TG No. 442E chemicals with negative results in the h-CLAT with log K_{OW} values greater than 3.5 should not be considered because chemicals with a log K_{OW} greater than 3.5 tend to produce false negative results (OECD, 2023b).

liquid paraffin (Narita et al., 2021; Narita et al., 2018). However, phthalic anhydride has yet to be tested in a modified h-CLAT. Ultimately, the rapid hydrolysis can explain the negative results in the available h-CLAT and most U-SENS assays. GARD Skin would be expected to have similar limitations to those of the other *in vitro* methods yet yielded a positive result.

Table 4-8. Summary of Phthalic Anhydride Data for KE 3 in Skin Sensitization AOP

Assay	Result	Remarks	Reference
h-CLAT	Negative	- Below 1.5-fold threshold for CD86 expression (EC_{150} not reported) [a] - Below 2-fold threshold for CD54 expression (EC_{200} not reported) [b] - Cell viability (IC50) measures not reported	(Bauch et al., 2012)
h-CLAT	Negative	- Below 1.5-fold threshold for CD86 expression (EC_{150} > 400 µg/mL) [a] - Below 2-fold threshold for CD54 expression (EC_{200} > 400 µg/mL) [b] - Cell viability (CV75) >400 µg/mL	(Nukada et al., 2012)
U-SENS	Negative	- EC_{150} and CV70 > 200 µg/mL[d,e]	(Piroird et al., 2015)
U-SENS	Negative	- Below 1.2-fold threshold for CD86 expression ($EC_{1.2}$ not reported) [c] - CV70 not reported	(Bauch et al., 2012)
U-SENS	Positive	- EC_{150} = 1,080 µM = 159.96 µg/mL [d] - Cell viability measures (*i.e.,* CV70 [d]) not reported; however, study authors state that SI values for CD86 were only determined in samples with cell viability >70%	(Natsch et al., 2013)
GARD Skin [f]	Positive	- Decision value (DV) = 3.60 (DV > 0 indicates a positive result) - Dose finding assays did not show cytotoxicity at 500 µM phthalic anhydride (concentration evaluated in main assay to determine sensitization)	(NTP, 2026)

GARD = Genomic Allergen Rapid Detection for assessment of skin sensitizers; h-CLAT = human cell line activation test; SI = stimulation index; U-SENS = Myeloid U937 Skin Sensitization Test
[a] EC_{150} = effective concentration showing the relative fluorescent intensity values of 150 in CD86 expression
[b] EC_{200} = effective concentration showing the relative fluorescent intensity values of 200 in CD54 expression
[c] $EC_{1.2}$ = the estimated concentrations showing the 120% SI of CD86 expression.
[d] EC_{150} = the estimated concentration showing the 150% SI of CD86 expression.
[e] CV70 = the estimated concentration showing 70% viability
[f] GARDskin assay provides qualitative results based on changes in gene expression (*i.e.,* sensitizer vs. non-sensitizer classification); however, this assay does not provide quantitative dose-response information.
Grey highlighting indicates studies considered *Acceptable* for use in quantitative dose-response in Section 4.2.1.3.

4.2.1.1.4 KE 4: T-cell Proliferation

KE 4 (T-cell proliferation) is evaluated *in vivo* using the local lymph node assay (LLNA), which is described by OECD TG No. 429 (Skin Sensitisation – LLNA) (OECD, 2010). Briefly, the LLNA evaluates T-cell proliferation in the auricular lymph nodes following topical application of a chemical to the ears of mice.

Phthalic anhydride has been evaluated in 5 LLNAs (Table 4-9), the majority of which were conducted prior to 2002 when OECD TG No. 429 was first adopted. However, the available LLNAs generally employed test methods consistent with OECD TG No. 429, with some deviations noted below in Table 4-9. Two of the available LLNAs contained deviations from the OECD guideline that may impact study results and interpretation, the most significant of which were the use of *in vitro* protocols for [^{3}H]TdR incorporation and lymphocyte proliferation, rather than through *in vivo* [^{3}H]TdR incorporation, as required by OECD TG 429. Therefore, these LLNAs (Arts et al., 2008; van Och et al., 2000) were considered *Acceptable* for qualitative use as part of the weight of scientific evidence below, and the

remaining three were *Acceptable* for quantitative use, and are therefore used in quantitative dose-response in Section 4.2.1.3.

Phthalic anhydride provided a positive result in all five LLNAs, with estimated EC_3 values (the estimated concentration needed to produce a stimulation index [SI] of 3) ranging from 0.16 to 0.36% in two studies (Dearman et al., 2000; van Och et al., 2000). Consistently, the LLNA by Plitnick et al. (2003) supports an EC_3 value between 0.15 (SI < 3) to 1.5% (SI > 3) phthalic anhydride; however, data were presented graphically only, and no EC_3 value was estimated. Similarly, the LLNA by Basketter et al. (1992) supports an EC_3 value of less than 2.5% phthalic anhydride (the lowest concentration tested [2.5%] produced an SI > 20). In a fifth LLNA, in which a single concentration of phthalic anhydride was evaluated as a positive control, a SI of approximately 93 was obtained in mice topically exposed to 25% phthalic anhydride (De Jong et al., 2009; Arts et al., 2008).

Table 4-9. Summary of Phthalic Anhydride LLNA Data for KE 4 in Skin Sensitization AOP

Reference	Brief Study Description	Result [a]
(Dearman et al., 2000)	Female BALB/c mice (4/group) exposed to 25 µL of vehicle (4:1 acetone/olive oil), 0.1, 0.25, 0.5, 1.0, 2.5% phthalic anhydride on both ears for 3 consecutive days. Following 2 rest days, mice were injected intravenously via the tail vein with 20 µCi of [^{3}H]TdR in 0.25 mL PBS. Five hours later auricular lymph nodes were excised, pooled, single cell suspensions prepared, and [^{3}H]TdR incorporation measured via β-scintillation counting.	Positive (EC_3 = 0.16%)
(van Och et al., 2000)	BALB/c mice (3/group) exposed to 25 µL of vehicle (4:1 acetone/olive oil), 0.25, 1, 2.5, 10, 25% phthalic anhydride on both ears for 3 consecutive days. Following 2 rest days, auricular lymph nodes were excised and pooled for each animal, single cell suspensions prepared and cultured with [^{3}H]TdR for 24 hours, and then [^{3}H]TdR incorporation measured via β-scintillation counting. Deviation(s) from OECD TG 429: - 3 animals/group (OECD TG 429 recommends at least 4) - *ex vivo* [^{3}H]TdR labeling (OECD TG 429 requires *in vivo* labeling via intravenous injection of [^{3}H]TdR through tail vein)	Positive (EC_3 = 0.36%)
(Plitnick et al., 2003)	Female BALB/c mice (5/group) exposed to 25 µL of vehicle (4:1 acetone/olive oil), 0.15, 1.5, 15% phthalic anhydride on both ears for 3 consecutive days. Following 1 rest day, mice were injected intravenously via the tail vein with 20 µCi of [^{3}H]TdR in 0.25 mL PBS. 5 hours later auricular lymph nodes were excised, pooled, single cell suspensions prepared, and [^{3}H]TdR incorporation measured via β-scintillation counting. Deviation(s) from OECD TG 429: - 1 rest day included (OECD TG 429 recommends 2 rest days)	Positive (0.15% < EC_3 < 1.5%)
(Basketter and Scholes, 1992)	CBA/Ca mice (4/group) exposed to 25 µL of vehicle (4:1 acetone/olive oil), 2.5, 5.0, 10% phthalic anhydride on both ears for 3 consecutive days. Following 1 rest day, mice were injected intravenously via the tail vein with 20 µCi of [^{3}H]TdR in 0.25 mL PBS. 5 hours later auricular lymph nodes were excised, pooled, single cell suspensions prepared, and [^{3}H]TdR incorporation measured via β-scintillation counting. Deviation(s) from OECD TG 429: - 1 rest day included (OECD TG 429 recommends 2 rest days)	Positive (EC_3 < 2.5%; SI ranged from 21−26% across groups)
(De Jong et al., 2009; Arts et al., 2008)	Male BALB/c mice (3/group) exposed to 25 µL of vehicle (4:1 acetone/olive oil) and 25% phthalic anhydride on both ears for 3 consecutive days. Following 2 rest days, auricular lymph nodes were excised and pooled for each animal,	Positive (EC_3 < 25%; SI = 93)

Reference	Brief Study Description	Result [a]
	single cell suspensions prepared and cultured with [^{3}H]TdR for 20−24 hours, and then [^{3}H]TdR incorporation measured via β-scintillation counting. Deviation(s) from OECD TG 429: - 3 animals/group (OECD TG 429 recommends at least 4) - *ex vivo* [^{3}H]TdR labeling (OECD TG 429 requires *in vivo* labeling via intravenous injection of [^{3}H]TdR through tail vein)	

[a] EC_3 = the estimated concentration needed to produce a SI of 3; SI = stimulation index is the ratio of the proliferation in treated groups to that in the concurrent vehicle control group
Grey highlighting indicates studies considered for use in quantitative dose response in Section 4.2.1.3.

4.2.1.1.5 Adverse Outcome: Inflammation Upon Challenge with Allergen

The adverse outcome, inflammation upon challenge with allergen, can be evaluated *in vivo* using the Buehler Test or the Guinea Pig Maximization Test (GPMT), both of which are described in OECD TG No. 406 (Skin Sensitisation) (OECD, 2022b), as well as using other methods such as skin prick testing. Additional evidence from human studies is also considered as part of the adverse outcome. Phthalic anhydride has been evaluated once in the GPMT, once in the Buehler test, once in a modified Buehler test, and via skin prick testing (Table 4-10). As discussed further below, phthalic anhydride gave a positive response for skin sensitization in all available studies.

Basketter et al. (1992) evaluated phthalic anhydride in the GPMT consistent with the method of Magnusson and Kligman (1969) and OECD TG No. 406. Briefly, Albino Dunkin Hartly guinea pigs (number of animals included not stated) were injected with a series of 6 intradermal injections of 0.1% phthalic anhydride in the shoulder region. Six to eight days post induction injections, an occluded patch containing 25% phthalic anhydride was placed at the injection site for 48 hours. Twelve to fourteen days after the induction patch was removed, animals were challenged by placing an occluded patch containing 10% phthalic anhydride on one flank for 24 hours. Skin was scored for erythema and oedema 24 and 48 hours after removal of the challenge patch. Ninety percent of guinea pigs gave a positive response for skin sensitization.

Botham et al. (2005) evaluated phthalic anhydride in standard and modified Buehler tests. The standard Buehler test was conducted consistent with OECD TG No. 406. Briefly, an occluded test patch containing vehicle or 20% phthalic anhydride was applied to the shaven left anterior flank of Hartly guinea pigs (10/sex/group) for 6 hours either 1 (standard test) or 3 (modified test) times a week for 3 weeks. For the modified Buehler test, the concentration of phthalic anhydride was reduced from 20 to 10% starting with the fifth application due to the severity of observed cutaneous reactions. Following the induction period, animals received no treatment for 11 days. For the challenge phase, which began 11 days after the last induction, animals treated with phthalic anhydride were cutaneously exposed to 20% phthalic anhydride on the shaven posterior right flank via occlusive dressing for six hours, while vehicle was applied to the shaven posterior left flank via occlusive dressing for six hours. Cutaneous reactions were then evaluated 24, 48, and 72 hours after the challenge. In the control group, a persistent discrete or moderate erythema (grade 1 or 2) was reported in 2 out of 10 animals (20%) on the right flank (acetone for induction; phthalic anhydride for challenge as a control), but not in the left flank (acetone for induction; acetone for challenge), which study authors speculated may be attributable to the irritating properties of phthalic anhydride. The standard and modified Buehler tests produced positive reaction rates in 17 out of 20 (85%) and 13 out of 20 (65%) animals, respectively, indicating a skin sensitization reaction.

Biagini et al. (1988) evaluated the sensitization potential of phthalic anhydride using the skin prick test in young male cynomolgus monkeys. Monkeys were split into 4 treatment groups, all of which received 10 consecutive weekly subcutaneous injections of 2 mg aluminum hydroxide, plus 10 weekly injections of one of the following: 200 µg of phthalic anhydride conjugated to monkey serum albumin (Group 1: phthalic anhydride-MSA); 200 µg of phthalic anhydride (Group 2: in ethanol-saline vehicle); 200 µg of MSA (Group 3); and (4) ethanol-saline alone (Group 4). Compared to other groups, monkeys injected with phthalic anhydride-MSA conjugate had significantly higher serum levels of phthalic anhydride-MSA conjugate-specific IgG on study weeks 4 through 10, as well as positive immediate skin tests (measured via cutaneous bluing reactions) after 8 weeks of injections. Although the route of administration via injection is not relevant for humans, this study provides data from a more human-related animal model and demonstrates sensitization potential of phthalic anhydride once it is able to enter the bloodstream.

Table 4-10. Summary of Phthalic Anhydride Data for the Adverse Outcome in the Skin Sensitization AOP

Assay	Result	Remarks	Reference
GPMT	Positive	- Positive skin response observed in 90% of animals	(Basketter and Scholes, 1992)
Buehler Test	Positive	- Positive skin reactions observed in 2/10 (20%) and 17/20 (85%) control and phthalic anhydride treated guinea pigs	(Botham et al., 2005)
Modified Buehler Test	Positive	- Positive skin reactions observed in 2/10 (20%) and 13/20 (65%) control and phthalic anhydride treated guinea pigs - Deviations from OECD TG 406: phthalic anhydride applied 3 times (instead of 1) per week to shaven flank for 3 consecutive weeks during induction phase	(Botham et al., 2005)
Skin Prick Test	Positive	- Male cynomolgus monkeys subcutaneous injected weekly with 2 mg aluminum hydroxide plus 200 µg of phthalic anhydride conjugated to monkey serum albumin for 10 consecutive weeks - Positive skin reactions observed on study weeks 8–10	(Biagnini et al., 1988)
GPMT = Guinea Pig Maximization Test			

4.2.1.1.6 Additional Evidence for Skin Sensitization in Humans

In addition to the studies discussed above that address specific KEs in the skin sensitization AOP, EPA identified evidence of allergic skin reactions in workers occupationally exposed to phthalic anhydride, including immediate and delayed onset allergic reactions, as described below. Immediate onset contact urticaria is mediated by an immunoglobulin (Ig) E (IgE) response, while delayed onset reactions are mediated by T-lymphocytes.

Evidence of allergic skin reactions comes mostly from case reports. OECD (2005) describes several early case reports of workers manufacturing phthalic anhydride and the results of patch testing. However, original copies of these studies were not reasonably available to EPA for independent review. OECD (2005) states:

> Two cases of urticarial rashes on exposed areas of the skin were found among workers at a chemical plant handling phthalic anhydride (Menschick, 1955).
>
> 191 workers were patch tested with the resin and hardeners, including phthalic anhydride at a plastics factory where epoxy resins, including maleic anhydride and phthalic

anhydride, were processed. An allergic response to a 0.1 % solution of phthalic anhydride in acetone was observed in 14% of the workers (Woyton et al., 1976).

More recently, Gutiérrez-Fernández et al. (2007) reported a case in a 43-year-old man working at a petrochemical refinery in Spain for 4 years with a 7-to-8-month history of immediate onset generalized urticaria and pruritus. Additional associated reported symptoms included ocular pruritus, rhinorrhea, dry cough, wheezing, and tightness of the chest. Total IgE and phthalic anhydride-specific IgE concentrations were 350 and greater than 100 IU/mL, respectively. The individual had positive skin prick tests to both phthalic anhydride (1%) dissolved in acetone and phthalic anhydride conjugated with human serum albumin (HSA) at a concentration of 10 mg/mL. Helaskoski et al. (2009) reported two additional case reports of contact urticaria. In the first case, the individual was exposed to phthalic anhydride in a warehouse where their job involved packaging of phthalic anhydride in powder form. In addition to urticaria, the individual also reported rhinitis and asthma symptoms, and had a positive skin prick test to phthalic anhydride with serum IgE levels of 1.4 kU/L. In the second patient, the individual was exposure to phthalic anhydride fumes released from welding painted pipes. In addition to contact urticaria, the patient also presented with rhinitis, and had a positive skin prick test to phthalic anhydride with serum IgE levels of 0.7 kU/L. Flaherty et al. (1988) describes a report in which a 43-year-old man with a 12-year history of exposure to phthalic anhydride and tetrachlorophthalic anhydride presented with contact urticaria, as well as wheezing, dyspnea, and coughing after exposure to either anhydride. The individual had positive intradermal and scratch tests to phthalic anhydride-HSA conjugate, but not tetrachlorophthalic anhydride-HSA.

Several case reports of allergic contact dermatitis have also been reported in consumers using nail polish containing phthalic anhydride, however, many of these case reports are confounded by the presence of trimellitic anhydride (TMA), a known dermal sensitizer. Moffitt and Sansom (2002) reported a 33-year-old woman with a 2-month history of intermittent itchy rash on the neck and around the eyes, with swelling of the eyelids. Positive reactions were observed in patch tests of her nail varnish and phthalic anhydride/TMA/glycols copolymer (1% pet.). Gach et al. (2005) describes four additional case reports of allergic contract dermatitis in which patch testing of phthalic anhydride (1% in butyl acetate) and phthalic anhydride/trimellitic anhydride/glycols copolymer (1% pet.) produced positive results in all four patients.

EPA received one submission via Section 8(e) from Carbide and Carbon Chemicals Corporation (1992) which focused on the description of human sensitization responses and asthma with workers exposed to phthalic anhydride but also included clinical manifestations of the skin. The report states that clinical manifestations of the skin consisted of skin irritation and "allergic type reactions have not been seen." However, the report also notes that "there have also been a few men who have not been able to tolerate phthalic anhydride at all. Several months ago, we had a man who developed widespread erythema following his initial contact… [he] possessed many physical characteristics which would predispose him to react to almost any irritant." There is not sufficient information in the report to differentiate between skin irritation and sensitization.

4.2.1.1.7 Additional Evidence for Sensitization in Animals

In addition to the studies discussed above that address specific KEs in the skin sensitization AOP, additional non-guideline *in vivo* studies of mice (10 studies) and monkeys (1 study) provide evidence of cytokine and antibody responses following dermal exposures to phthalic anhydride that are consistent with skin sensitization and an immune response. Observed effects include consistent increases in serum IgE and IgG levels in studies of mice that adhere to an induction/challenge paradigm, and some evidence that dermal exposure to phthalic anhydride may primarily induce a type 2 (Th2) cytokine (*e.g.,* IL-4, IL-5, IL-10, and IL-13) response, over a type 1 (Th1) cytokine (*e.g.,* IFN-$_{\gamma}$ [interferon gamma], IL-

2, and IL-12) response. These data are also further considered in the respiratory sensitization AOP (Section 4.3.1.1).

The aforementioned study by Biagnini et al. (1988) evaluated the immune response in male cynomolgus monkeys). Total serum IgE and phthalic anhydride-specific IgE levels were unaffected, whereas phthalic anhydride-specific IgG levels were significantly elevated in monkeys administered phthalic anhydride-monkey serum albumin (group 1) after 4, 6, 8, and 10 weeks of treatment, compared to other treatment groups.

Antibody responses have also been observed in mice dermally exposed to phthalic anhydride. Dearman et al. (1992) evaluated serum IgE levels in female BALB/c mice administered 50 µL of vehicle or 25% phthalic anhydride to each shaved flank (induction), followed by an application of 25 µL of 25% phthalic anhydride to the dorsum of both ears 7 days later (challenge). Compared to controls, treatment with phthalic anhydride increased serum IgE levels in mice 8, 14, and 21 days after the initial induction exposure. In another study by the same group, female BALB/c mice were administered 50 µL of 0, 1, 5, 10, 25, or 50% phthalic anhydride bilaterally on the shave flanks (induction), followed by 25 µL of 0, 1, 5, 10, 25, or 50% phthalic anhydride to the dorsum of both ears 7 days later (challenge) (Dearman and Kimber, 1992). Animals in the 1, 5, 10, and 50% treatment groups were used to determine serum IgG antibody levels, while animals in the 25% group were used to determine serum IgE and IgG isotype distribution. Serum IgG anti-hapten antibody levels were significantly increased in mice treated with 5, 10, and 50% phthalic anhydride 8, 14, and 21 days after the initial induction exposure, while treatment with 25% phthalic anhydride significantly increased serum IgE, IgG1, and IgG2b antibody responses.

Similar results were obtained in a study by Ban et al. (2005) in which female BALB/c mice were administered 50 µL of 0 or 12.5% phthalic anhydride dermally to both shaved flanks for 4 consecutive days (induction), followed by dermal application of 0 or 6.25% phthalic anhydride to the dorsum of both ears on day 7 (challenge). Four days after the challenge, in the phthalic anhydride treatment group, significant increases in serum IgE (but not IgG2a) were observed, and *ex vivo* splenocyte production of Th2 cytokines IL-4 and IL-10 were increased, while Th1 cytokines IL-2 and IFN-$_\gamma$ was unaffected.

Three studies of similar design with BALB/c mice provide additional evidence of a cytokine response following dermal exposure to phthalic anhydride. In the first study, Dearman et al. (2000) investigated cytokine production in BALB/c mice administered 50 µL of a 1 molar solution of phthalic anhydride bilaterally on each shaved flank. Five days later the treatment was repeated (induction) and then another 5 days later 25 µL of phthalic anhydride or vehicle was applied to the dorsal side of both ears daily for 3e consecutive days (challenge). Thirteen days after the first induction exposure, draining auricular lymph nodes were excised and single-cell suspensions were prepared. Treatment with phthalic anhydride resulted in a mixed response of increased Th1 cytokine IL-12 (but not IFN-$_\gamma$) and Th2 cytokine IL-10 (but not IL-4). In the second study, Plitnick et al. (2003) administered 100 µL of 2.5 and 15% phthalic anhydride on the shaved flanks of female BALB/c mice on study days 0 and 5 (induction), and then administered 12.5 µL of 2.5 of 15% phthalic anhydride to each side of both ears on study days 10, 11, and 12 (challenge). Two days later (study day 14), mice were sacrificed, auricular lymph nodes were excised, and mRNA levels of cytokines were determined. No effect was observed in mice administered 2.5% phthalic anhydride, while Th2 cytokines IL-4, IL-10, and IL-13 mRNA was significantly increased in mice administered 15% phthalic anhydride and mRNA for the Th1 cytokine, IFN-$_\gamma$ was unaffected. In a third study, male and female BALB/c mice were sensitized with 25 µL phthalic anhydride (25%) or vehicle for 3 consecutive days via dermal application to the dorsum of both ears, and then 3 days later the auricular lymph nodes were excised and evaluated (Vandebriel et al., 2000). Treatment with phthalic anhydride resulted in increased weight of the auricular lymph nodes,

increased lymphocyte proliferation, and increased levels of Th1 cytokine, IFN-$_\gamma$ and Th2 cytokine, IL-4 mRNA.

Sung et al. (2016) investigated dermal phthalic anhydride exposure in young (2-month-old) and old (12-month-old) IL-4/Luc/CNS-1 transgenic mice, which express luciferase under the control of the IL-4 promoter. Mice were dermally exposed to vehicle or 100 µL of 15% phthalic anhydride on the dorsum and shaved back three times per week for two weeks. Observed effects included: decreased absolute thymus weight in young and old mice; increased absolute mesenteric lymph node and spleen weight in young, but not old mice; increased ear thickness and thickness of the epidermis and dermis of both young and old mice; increased serum IgE, as well as number of mast cells, IL-6, and vascular endothelial growth factor (VEGF) in ear homogenates from young and old mice; and increased luciferase expression (as determined by signal intensity) in mesenteric lymph node, and pancreas, indicating increased expression of the Th2 cytokine IL-4 in both young and old mice.

Three publications from the same research group provide additional evidence of an allergic skin reaction in mice (Bae et al., 2013; Bae et al., 2011; Bae et al., 2010). In the first study, female C57BL/6 and BALB/c were dermally exposed to 40 µL of vehicle or 5% phthalic anhydride on the dorsum of both ears and shaved back three times per week for 4 weeks (Bae et al., 2010). In both strains of mice, treatment with phthalic anhydride resulted in increased ear thickness; allergic skin inflammation; increased absolute auricular lymph node weight; increased serum IgE levels; and increased levels of cytokines (*e.g.,* IL-6 and VEGF). Notably, the response was higher in BALB/c mice compared to C57BL/6, indicating a strain difference in sensitivity. In the second study, wild-type and transgenic mice over-expressing human GATA3 (GATA binding protein 3) were dermally exposed to 50 µL of vehicle or 1, 5, and 10% phthalic anhydride on the dorsum of both ears three times a week for 3 weeks (Bae et al., 2011). Treatment with phthalic anhydride resulted in increased ear thickness and increased auricular lymph node weight in wild-type (>5%) and transgenic mice (>1%), and increased serum Th2 Ig levels of IgE and IgG1 (but not Th1 type IgG2a or IgG3) in both strains at all dose levels. Additionally, secretion of the Th2 cytokines IL-4, IL-5, and IL-13 was generally increased in both strains of mice, while secretion of the Th1 cytokine IFN-$_\gamma$ was suppressed. Observed responses were generally higher in transgenic mice compared to wild-type mice sugging a role for GATA-3 in the observed allergic skin inflammation in mice. Finally, in a third study, female wild-type and Krüppel-like factor 10 (*KLF-10*) deficient mice (KLF10$^{-/-}$) were dermally exposed to 40 µL of vehicle or 1 and 5% phthalic anhydride on the dorsum of the ear and shaved back 3 times per week for 3 weeks (Bae et al., 2013). Ear thickness and auricular lymph node weight was increased in wild-type and KLF10$^{-/-}$ mice of both dose groups; epidermis thickness and the number of infiltrated mast cells were increased in high-dose wild-type and KLF10$^{-/-}$ mice; and phosphorylated ERK (5%), protein kinase c delta (PKC-δ) (≥1%), and IL-6 (≥1%) were increased in ear homogenate from both wild-type and KLF10$^{-/-}$ mice. Notably, responses were more pronounced in KLF10$^{-/-}$ mice compared to wild-type mice sugging a role for GATA-3 in allergic skin inflammation in mice.

4.2.1.2 Evidence Integration Conclusions: Skin Sensitization

Consistent evidence of skin sensitization for phthalic anhydride comes from human data, as well as *in chemico*, *in vitro*, and *in vivo* experimental animal data. As discussed in Section 4.2.1.1.6, EPA identified some evidence of allergic skin reactions in consumers and workers occupationally exposed to phthalic anhydride, including immediate and delayed onset allergic reactions.

Phthalic anhydride showed moderate reactivity in two *in chemico* DPRA assays demonstrating potential to covalently interact with skin proteins (KE 1 in skin sensitization AOP as defined in OECD TG No. 497) (Section 4.2.1.1.1). The mechanism by which phthalic anhydride covalently interacts with proteins

has been proposed to be through protein acylation, which is a reaction in which the electrophilic acyl group preferentially reacts with nucleophiles, such as lysine residues (Johnson et al., 2022; Wareing et al., 2017; Piroird et al., 2015; Bauch et al., 2012). Consistent with this mechanism, phthalic anhydride preferentially depleted synthetic lysine peptides in both DPRA assays (Section 4.2.1.1.1). Additionally, dermal application of phthalic anhydride caused consistent dose-related T-cell proliferation in five LLNA assays (KE 4 in skin sensitization AOP as defined in OECD TG No. 497) (Section 4.2.1.1.4), with EC3 values ranging from 0.16 to 0.36% phthalic anhydride (Section 4.2.1.1.4). Phthalic anhydride also tested positive in one GPMT, one Buehler test, and one modified Buehler test demonstrating inflammation upon challenge with phthalic anhydride (Section 4.2.1.1.5). In addition, evidence from 10 non-guideline studies of mice and 1 study of monkeys, provide consistent evidence of cytokine and antibody responses following dermal exposures to phthalic anhdride that are consistent with skin sensitization and an immune response (Section 4.2.1.1.6).

Phthalic anhydride was negative for sensitization in several *in vitro* assays for KE 2 (keratinocyte cellular responses, Section 4.2.1.1.2) and KE 3 (DC responses, Section 4.2.1.1.3), including 2 KeratinoSens and 1 LuSens assays for KE 2. Results were mixed for KE 3 assays, where phthalic anhydride tested negative in 2 h-CLAT assays, was positive in 1 of 3 U-SENS assays and was positive in one GARDskin assay. Although these results appear to be inconsistent with phthalic anhydride being a skin sensitizer, these inconsistencies may be explained by the fact that phthalic anhydride rapidly hydrolyzes to *o*-phthalic acid (a non-sensitizer) in aqueous solutions, with hydrolysis half-lives ranging from approximately 30 to 90 seconds (U.S. EPA, 2026h). Rapid hydrolysis of phthalic anhydride to *o*-phthalic acid in aqueous cell culture medium may lead to false negative results in KE 2 and KE 3 assays where hydrolysis may occur. Additionally, as discussed in OECD TG No. 442D, chemicals that show exclusive reactivity towards lysine-residues may show negative results in both the KeratinoSens and LuSens assays (OECD, 2024). This is because the mechanism leading to activation of the Keap1-Nrf2-ARE pathway in both assays involves the interaction of electrophilic chemicals with nucleophilic thiols in cysteine residues of Keap-1, and phthalic anhydride preferentially interacts with lysine in the DPRA.

Overall, EPA considers there to be robust evidence to support the conclusion that phthalic anhydride is a skin sensitizer and skin sensitization is considered further for dose-response assessment in Section 4.2.1.3. Notably, EPA's conclusion is consistent with that of other regulatory and authoritative bodies. OECD (2005), Health Canada (2019), Australia NICNAS (2013), and ACGIH (2025) have also identified phthalic anhydride as a skin sensitizer.

4.2.1.3 Dose-Response Assessment: Skin Sensitization

Based upon available human and animal data, EPA identified sensitization as the key endpoint for dermal POD derivation. Phthalic anhydride is a well-documental dermal sensitizer and EPA has determined that skin sensitization is the most sensitive non-cancer effect of dermal exposure for which data are available. An approach to quantifying risk from exposure to products containing dermal sensitizing pesticide chemicals that do not bear labels was developed by EPA for assessment of risk from exposure to treated wood (U.S. EPA, 2004). EPA used a quantitative approach to assess the risk to isothiazolinone biocides for skin sensitization (U.S. EPA, 2020b) utilizing both *in vitro* data and *in vivo* human and animal studies. For formaldehyde, EPA also used a quantitative approach to assess the risk for skin sensitization based on human patch test studies (U.S. EPA, 2024). These previous assessments provide precedent in EPA for deriving and using PODs based on sensitization from phthalic anhydride exposure as presented below.

EPA used the Skin Allergy Risk Assessment – Integrated Chemical Environment (SARA-ICE) model for deriving a quantitative POD for skin sensitization. The SARA-ICE model was developed through

collaboration between NICEATM and Unilever. The SARA-ICE POD model was evaluated by OECD for inclusion in Test Guideline (TG) 497, the DAs for Skin Sensitization (TG 497) (OECD, 2025b). SARA-ICE is a Bayesian statistical model, built using a database of 434 chemicals with skin sensitization data from *in vitro* and *in vivo* information sources. These sources include: the DPRA, the kDPRA, the h-CLAT, the U-SENS, the KeratinoSens, human predictive patch tests (HPPT), and the LLNA (Reinke et al., 2025; Reynolds et al., 2019). All of these methods address the different KEs of the skin sensitization AOP (Figure 4-1). Across the database of 434 chemicals underlying the SARA-ICE model, the most common sensitization rate in HPPT data used to train and validate the model was 1 in 100 (1%) response, which is why the SARA-ICE model was validated for estimating the ED_{01}. Therefore, the 1% response rate has the least model uncertainty because this response rate is informed by the most data. From this statistical model, an ED_{01}, an estimate of the applied HPPT dermal dose ($\mu g/cm^2$) at which there is a 1% chance of inducing sensitization is derived. The model provides a probability distribution that describes the plausible values of the ED_{01}, which are conditional on the chemical-specific input data. Based on the distribution produced, a measure of certainty can be understood, exhibited by the spread of the uncertainty around the geometric mean (a wider distribution indicates less certainty in the data). TG 497 derives a POD from the distribution, which is the geometric mean of the distribution. The geometric mean is the centralized HPPT dose derived from the ED_{01} distribution. This is the "most likely estimate" of the ED_{01}. The 5th percentile is the HPPT dose with a 95% chance of being less than the ED_{01}. It is unlikely that the ED_{01} will be any lower than this dose (*e.g.,* the model provides a confidence with 95% certainty that the ED_{01} is greater than this value). Thus, the model estimates the ED_{01} as a probability distribution summarized as a 90% centered interval between the 5th to 95th percentiles, and the model provides confidence with 90% certainty that the ED_{01} is within this range.

In collaboration with NICEATM, the SARA-ICE model was used to evaluate phthalic anhydride for a dermal POD. The SARA-ICE model is publicly available through NTP (https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/skin-sens/da/SARA-ICE). As outlined in Table 4-11, data from 2 DPRAs (KE 1), 1 kDPRA (KE 1), 2 KeratinoSens assays (KE 2), 2 h-CLAT assays (KE 3), 2 U-SENS assays (KE 3), and 5 LLNAs (KE 4) were initially considered for inclusion in the SARA-ICE model (Wareing et al., 2017; Natsch et al., 2013; Bauch et al., 2012; Nukada et al., 2012; Arts et al., 2008; Plitnick et al., 2003; Dearman et al., 2000; van Och et al., 2000; Basketter and Scholes, 1992). Data from 1 KeratinoSens assay (Bauch et al., 2012), 1 h-CLAT assay (Bauch et al., 2012), and 2 U-SENS assays (Natsch et al., 2013; Bauch et al., 2012) were excluded because study authors did not report all requisite data points for each endpoint required for inclusion in the SARA-ICE evaluation (*i.e.,* IC_{50}, CV75, and CV70 values were not reported for KeratinoSens, h-CLAT, and U-SENS assays, respectively). For the remaining studies, EPA developed DERs, which are available in U.S. EPA (2026b). All of the remaining assays were deemed to be of acceptable quality for inclusion in the SARA-ICE evaluation, with the exception of 1 DPRA (Bauch et al., 2012) and 2 LLNAs (Arts et al., 2008; van Och et al., 2000), which were excluded from the SARA-ICE evaluation. As noted above, the DPRA reported by Bauch et al. was not acceptable for inclusion in the SARA-ICE evaluation because the peptide to phthalic anhydride ratio was 1:15 for synthetic cysteine peptides and 1:3 for synthetic lysine peptides, whereas OECD TG No. 442C requires ratios of 1:10 and 1:50 for synthetic cysteine and lysine peptides to test substance, respectively (OECD, 2023a). Similarly, LLNAs by van Och et al. and Arts et al. were considered not acceptable for inclusion in the SARA-ICE evaluation because following exposure to phthalic anhydride, lymphocyte cells were isolated from the auricular lymph nodes and labelled with [^{3}H]TdR *ex vivo*. In contrast, OECD TG No. 429 requires *in vivo* labeling of proliferating lymphocytes intravenous injection of [^{3}H]TdR through tail vein (OECD, 2010). Ultimately, data from 1 DPRA (Gerberick et al., 2004), 1 kDPRA (Wareing et al., 2017), 1 KeratinoSens assay (Natsch et al.,

2013), 1 h-CLAT assay (Nukada et al., 2012), and 3 LLNAs (Plitnick et al., 2003; Dearman et al., 2000; Basketter and Scholes, 1992) were included in the quantitative SARA-ICE evaluation (Table 4-11).

Table 4-11. Summary of Phthalic Anhydride Data Included in the SARA-ICE Model

KE 1			KE 2		KE 3			KE 4
DPRA (% Depletion)		kDPRA ($M^{-1}s^{-1}$)	KeratinoSens		h-CLAT			LLNA (EC3, %)
Cysteine	Lysine		$EC_{1.5}$ (µM)	IC_{50} (µM)	CD54, EC_{200} (µg/mL)	CD86, EC_{150} (µg/mL)	CV_{75} (µg/mL)	
1.9 [a]	75[a]	–0.67[b]	>2,000[g]	>2,000[g]	>400[c]	>400[c]	>400[d]	0.16[d] <1.5[e] <2.5[f]

Abbreviations: DPRA = Direct Peptide Reactivity Assay; h-CLAT = Human Cell Line Activation Test; kDPRA = Kinetic DPRA; KE = Key event; LLNA = local lymph node assay; NR = Not reported; CV70 = the estimated concentration showing 70% viability

[a] (Gerberick et al., 2004)
[b] (Wareing et al., 2017)
[c] (Nukada et al., 2012)
[d] (Dearman et al., 2000)
[e] (Plitnick et al., 2003)
[f] (Basketter and Scholes, 1992)
[g](Natsch et al., 2013)

The SARA-ICE model can run an infinite number of samples to estimate the ED_{01}, however, for efficiency, a default of 10,000 samples is used to make the estimates. Repeat runs of the model will have slight variation but will not change overtly. EPA ran the SARA-ICE model using two separate sets of input data. The first one included all data in Table 4-11). The second dataset excluded all h-CLAT and KeratinoSens data due to a concern that phthalic anhydride may have hydrolyzed to *o*-phthalic acid in cell culture media, thereby resulting in a false negative response in *in vitro* assays for KE 2 and KE 3. Additionally, based on DPRA assay results, phthalic anhydride preferentially binds to lysine residues and as discussed in OECD TG 442D, chemicals that show exclusive reactivity towards lysine-residues may show negative results in the KeratinoSens assay (OECD, 2024). Since the available U-SENS data did not report all requisite data (*i.e.,* missing CV70), the second run therefore excludes all KE 2 and KE 3 data.

SARA-ICE model distribution outputs are shown in Figure 4-2, while model results are shown in Table 4-12. The OECD TG 497 model predicted an ED_{01} (geometric mean) of 240 µg/cm^2 when all available data were considered. The TG 497 model predicted an ED_{01} of 45 µg/cm^2 when the potential false negative data from h-CLAT and KeratinoSens assays were removed. Based on the SARA-ICE model, phthalic anhydride is predicted to be a skin sensitizer with confidence and the estimate of the sensitizing level for 1% of an HPPT eligible population ranging from 45 to 240 µg/cm^2.

There is some uncertainty associated with the SARA-ICE estimated ED_{01} values. Uncertainty in the ED_{01} is reflected in the SARA-ICE prediction uncertainty ratio (SPUR), which is measured by the ratio of the 50th percentile and the 5th percentile. As discussed in the SARA-ICEDA within OECD TG No. 497 (OECD, 2025b), "a minimum of 2 test methods per compound, covering 2 separate KEs from within OECD TG 442C, 442D, or 442E" should be used as an input to ensure a higher confidence estimate. However, a greater number of data types and inputs will likely also increase confidence of the

model ED_{01} output depending on the concordance of the additional data utilized. As shown in Figure 4-2 and Table 4-12, there is less variance in the model run that excludes KE2 and KE3 data that were part of the first run, as demonstrated by the lower SPUR of 17 (vs. a SPUR of 22 when all data are included). Therefore, there is less uncertainty with the ED_{01} of 45 µg/cm^2.

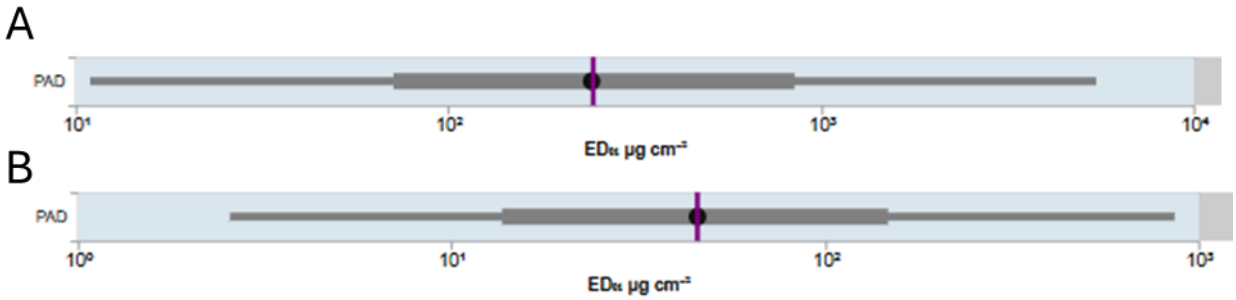

Figure 4-2. SARA-ICE Output Distributions
ED_{01} estimates represented as centered 90% credible intervals (thin line), 50% credible intervals (thick line) and median (bullet). **A)** OECD Guideline 497 POD distribution with all data, the purple line indicates the POD (geometric mean). **B)** OECD Guideline 497 POD distribution without KeratinoSens and h-CLAT data, the purple line indicates the POD (geometric mean). Note the scale difference.

Table 4-12. SARA-ICE Model Results

Model Run	POD (ED_{01} Geometric Mean) (µg/cm^2)	ED_{01} 5th Percentile (µg/cm^2)	ED_{01} 50th Percentile (µg/cm^2)	ED_{01} 95th Percentile (µg/cm^2)	SPUR (ED_{01} 50th divided by the ED_{01} 5th)
Phthalic Anhydride (All Data)	240	11	240	5,500	22
Phthalic Anhydride (KE 2 and 3 Data Excluded)	45	2.6	45	860	17

POD = Point of departure; ED_{01} = estimate of the applied HPPT dermal dose (µg/cm^2) at which there is a 1% chance of inducing sensitization; SPUR = SARA-ICE prediction uncertainty ratio.

The SARA-ICE model was trained and validated as described in OECD TG 497 (OECD, 2025b) to predict the ED_{01}, or the likelihood of a dose sensitizing 1% of the population. EPA also considered the distribution at different estimates (*i.e.,* ED_{05}, ED_{10}, and ED_{20}), which is described in more detail in Appendix D. Briefly, EPA utilized the SARA-ICE extended model (Reinke et al., 2026) to determine the geometric mean of the distributions for additional EDx distributions including ED_{05}, ED_{10}, and ED_{20} where "x" is equal to the estimate of the applied HPPT dermal dose (µg/cm^2) at which there is an x% chance of inducing sensitization. EPA used the same input data as above for a total of six additional runs (*i.e.,* ED_{05}, ED_{10}, and ED_{20} with inputs for "All data" and "KE 2 and KE 3 Data Excluded." The results are summarized in Table 4-13. The geometric mean of the ED_{05} was slightly larger than the geometric mean of the ED_{01} for the model runs with "All data" (240 µg/cm^2 for ED_{01} compared to 380 µg/cm^2 for ED_{05}) as well as for model runs with "KE 2 and 3 Data Excluded" (45 µg/cm^2 for ED_{01} compared to 65 µg/cm^2 for ED_{05}). The geometric mean of the ED_{10} was approximately 2 to 2.5 times the geometric mean of the ED_{01} for the model runs with "All data" (240 µg/cm^2 for ED_{01} compared to 620 µg/cm^2 for

ED_{10}) as well as for model runs with "KE 2 and 3 Data Excluded" (45 µg/cm^2 for ED_{01} compared to 110 µg/cm^2 for ED_{10}). The geometric mean of the ED_{20} was approximately 7 times the geometric mean of the ED_{01} for the model runs with "All data" (240 µg/cm^2 for ED_{01} compared to 1700 µg/cm^2 for ED_{20}) as well as for model runs with "KE 2 and 3 Data Excluded" (45 µg/cm^2 for ED_{01} compared to 290 µg/cm^2 for ED_{20}). Currently, the SARA-ICE extended model has not been validated for estimating EDx values other than 1%, nor does the extended model report the 5th, 50th, or 90th percentile EDx estimates, so SPUR values for EDx estimates other than 1% cannot be estimated. There is a non-linear relationship between the EDx distributions.

Table 4-13. SARA-ICE Extended Model Results for Additional EDx Distributions

Model Run	EDx	POD Geometric Mean (µg/cm^2)	EDx 5th Percentile	EDx 50th Percentile	EDx 95th Percentile
Phthalic Anhydride (All Data) [a]	01	240	11	240	5,500
Phthalic Anhydride (All Data)	05	380	– [d]	–	–
Phthalic Anhydride (All Data)	10	620	–	–	–
Phthalic Anhydride (All Data)	20	1,700	–	–	–
Phthalic Anhydride (KE 2 and 3 Data Excluded) [b]	01	45	2.6	45	860
Phthalic Anhydride (KE 2 and 3 Data Excluded)	05	65	–	–	–
Phthalic Anhydride (KE 2 and 3 Data Excluded)	10	110	–	–	–
Phthalic Anhydride (KE 2 and 3 Data Excluded)	20	290	–	–	–
Phthalic Anhydride (KE 2 and 3 Data & 2 LLNA Results Excluded) [c]	01	50	2.5	48	1,000
Phthalic Anhydride (KE 2 and 3 Data & 2 LLNA Results Excluded) [c]	05	73	–	–	–
Phthalic Anhydride (KE 2 and 3 Data & 2 LLNA results Excluded) [c]	10	120	–	–	–
Phthalic Anhydride (KE 2 and 3 Data & 2 LLNA results Excluded) [c]	20	330	–	–	–

Abbreviations: POD = point of departure; EDx = Geometric Mean of the EDx Distribution (µg/cm²) where x is percent of population sensitized; KE = key event;
[a] "Phthalic Anhydride (All Data)" indicates SARA-ICE inputs of 1 DPRA assay; 1 KeratinoSens; 1 h-CLAT; 3 LLNAs
[b] "Phthalic Anhydride (KE 2 and 3 Data Excluded)" indicates SARA-ICE inputs of 1 DPRA assay; 3 LLNAs

Model Run	EDx	POD Geometric Mean (µg/cm²)	EDx 5th Percentile	EDx 50th Percentile	EDx 95th Percentile

[c] "Phthalic Anhydride (KE 2 and 3 Data Excluded & 2 LLNA Results Excluded)" indicates SARA-ICE inputs of 1 DPRA assay; 2 LLNAs
[d] Dash indicates that values were not available; the SARA-ICE extended model outputs did not include the 5th and 95th percentiles.

EPA considered the application of the additional EDx estimates from the SARA-ICE extended model (Table 4-13), to extrapolate to EDx values below 1% using the geometric means from the ED_{01}, ED_{05}, ED_{10}, and ED_{20} distributions. This approach allowed approximation of the geometric mean (µg/cm²) of the distribution at which the percent chance of inducing sensitization approaches zero, which could be used to inform the range in variability of the allergic response across the human population associated with exposure to phthalic anhydride. These values were selected to demonstrate a rough estimate of the protectiveness of the POD if additional UFs were applied as described in Appendix D. At an effective dermal hazard value of 4.5 µg/cm² (*i.e.,* 45 µg/cm² divided by a total uncertainty factor of 10×), the EDx is over 99.99%. EPA also included an estimate for the effective dermal hazard value of 15 µg/cm² (*i.e.,* 45 µg/cm² divided by a total uncertainty factor of 3×). The EDx was over 99.99% for this as well.

The SARA-ICE model was trained and validated specifically on the ED_{01}, and therefore there is additional uncertainty associated with the use of other EDx values. The full distribution, including the 5th and 95th percentiles, is not yet publicly available on the web application of the SARA-ICE model, as stated in the footnotes of Table 4-13above. Given the fact that the SARA-ICE model is trained and validated on the ED_{01} estimate (OECD, 2025b), and the uncertainties associated with the ED_{05}, ED_{10}, and ED_{20}, results, EPA preliminarily concluded that the best available science supports the selection of a POD from the ED_{01} distribution above these other estimates.

For input into the draft risk evaluation of phthalic anhydride, EPA selected the ED_{01} of 45 µg/cm² as the POD (Table 4-12). This POD was selected over the ED_{01} of 240 µg/cm², because suspected false negative data from the h-CLAT and KeratinoSens assays due to hydrolysis in aqueous solution were removed from the analysis. For use in the draft risk evaluation for phthalic anhydride, EPA is proposing a UF_H of 1× to the POD for phthalic anhydride based on the high degree of refinement and confidence in the POD, which reflects the geometric mean of the ED_{01} estimate and therefore is intended to be protective of 99% of the population. Application of a UF_H of 1× represents a chemical-specific decision, application of a UF_H other than 1× may be supported when SARA-ICE is applied for other chemicals.

The POD is anticipated to encompass much of the variability across sensitized individuals in the magnitude of the response. EPA acknowledges there may be residual population variability. For instance, the POD is intended to protect against induction of new cases of sensitization in 99% of the population, but may not be protective of the elicitation phase, which reflects individuals who are already sensitized because elicitation thresholds will generally be lower than the induction thresholds (Griem et al., 2003; Scott et al., 2002). Uncertainty in the POD (*i.e.,* in the geometric mean estimate of the dose at which 1% of the population is sensitized) is reflected in the SPUR of 17. The resulting uncertainty in the proposed dermal POD for phthalic anhydride is expected to be more refined compared to previous EPA assessments that have not used the novel SARA-ICE model. Indeed, OPPT previously applied a UF_H of 10× for skin sensitization in the formaldehyde risk evaluation (U.S. EPA, 2024)and EPA's Office of Pesticide Programs (OPP) applied a UF_H of 10× for skin sensitization for the isothiazolinone biocides (U.S. EPA, 2020b). EPA is soliciting comments from the SACC on the strengths and uncertainties of EPA's use of the SARA-ICE model to derive its draft POD.

4.2.1.4 Weight of Scientific Evidence Conclusions: Skin Sensitization POD

EPA has preliminarily selected the ED_{01} of 45 µg/cm^2, which is the applied HPPT dermal dose at which there is 1% chance of inducing sensitization, for calculation of risk from dermal exposures to phthalic anhydride. A total UF of 1× was selected for use as the benchmark MOE (based on a UF_H of 1× , which was reduced from 10× because the SARA-ICE model for phthalic anhydride accounts for variability in the human population). The draft POD is supported by the following weight of scientific evidence considerations:

- There is some evidence of allergic skin reactions in consumers and workers dermally exposed to phthalic anhydride, including immediate and delayed onset allergic reactions (Section 4.2.1.1.6).
- Phthalic anhydride tested positive for sensitization in a variety of *in chemico, in vitro*, and *in vivo* assays across KEs in the skin sensitization AOP (Section 4.2.1.1). Phthalic anhydride tested positive for sensitization in two DPRAs and one kDPRA (KE 1; Section 4.2.1.1.1); one GARDskin assay (KE 3; Section 4.2.1.1.3); five LLNAs (KE 4; Section 4.2.1.1.4); and one GPMT and two Buehler tests (adverse outcome; Section 4.2.1.1.5).
- Several inconsistencies were observed across available KE 2 and KE 3 assays. For example, phthalic anhydride was negative for sensitization in two *in vitro* KeratinoSens assays and one *in vitro* LuSens assay (KE 2; Section 4.2.1.1.2); and two *in vitro* h-CLAT assays and two of three *in vitro* U-SENS assays (KE 3; Section 4.2.1.1.3). However, these inconsistencies may be explained by the rapid hydrolysis (hydrolysis $t_{1/2}$ = 30–90 seconds in aqueous solution) of phthalic anhydride to *o*-phthalic acid (non-sensitizer) in aqueous cell culture medium. Additionally, based on DPRA results, phthalic anhydride preferentially covalently interacts with lysine peptides. As discussed in OECD TG No. 442D, chemicals that show exclusive reactivity towards lysine-residues may show negative results in both the KeratinoSens and LuSens assays (OECD, 2024). This is because the mechanism leading to activation of the Keap1-Nrf2-ARE pathway in both assays involves the interaction of electrophilic chemicals with nucleophilic thiols in cysteine residues of Keap-1.
- The POD is derived using the SARA-ICE model, which integrates results from multiple assays to predict the applied HPPT dermal dose at which there is 1% chance of inducing sensitization. Data from one DPRA (KE 1), one kDPRA (KE 1), and three LLNAs (KE 3) of phthalic anhydride were included in the SARA-ICE evaluation to estimate the applied dermal POD of 45 µg/cm^2.
- One uncertainty associated with the SARA-ICE estimated ED_{01} is that only *in chemico* DPRA, kDPRA, and *in vivo* murine LLNA data were suitable for inclusion in the evaluation. No HPPT data were available for phthalic anhydride. There is some uncertainty in extrapolating from murine potency to human potency, and this uncertainty is reflected in the widths of the 90% credible intervales used to represent the estimate of the ED_{01} (90% credible interval: 2.6, 860 µg/cm^2).
- Phthalic anhydride is classified (GHS) as Skin Sens. 1 (H317: May cause an allergic skin reaction) in the EU. Additionally, OECD (2005), Health Canada (2019), Australia NICNAS (2013), and ACGIH (2025) have also concluded that phthalic anhydride is a skin sensitizer. Note that these groups did not derive PODs for skin sensitization or quantify dermal risk.

4.2.2 Phthalic Acid

EPA evaluated reasonably available information pertaining to the human health effects of dermal exposure to *o*-phthalic acid. Although abundant evidence supports the conclusion that phthalic anhydride is a dermal sensitizer, there is no evidence that *o*-phthalic acid is also a sensitizer.

Available mechanistic NAMs data support the conclusion that *o*-phthalic acid is not a dermal sensitizer. NICEATM (2026) recently evaluated phthalic anhydride and *o*-phthalic acid in the GARDskin assay (KE 3 in skin sensitization AOP) consistent with OECD TG 442E. Under the conditions of the study, *o*-phthalic acid was considered to be a non-sensitizer. EPA concludes that *o*-phthalic acid is not a dermal sensitizer. There is no evidence to indicate route-specific toxicity for *o*-phthalic acid.

EPA did not derive a dermal hazard value for *o*-phthalic acid because there was no quantitative dermal characterization of risk from dermal exposures to *o*-phthalic acid in the general population assessment, as further discussed in the general population screening assessment (U.S. EPA, 2026f) and above in Section 3.3. Briefly, physical and chemical properties of *o*-phthalic acid indicate that dermal absorption is not expected.

4.3 Inhalation Exposure

Reasonably available information pertaining to the human health effects of inhalation exposure to phthalic anhydride or its immediate hydrolysis product, *o*-phthalic acid, are provided in Section 4.3.1 and 4.3.2, respectively.

4.3.1 Phthalic Anhydride

EPA identified data from humans, *in vivo* experimental animal studies, and *in vitro* studies that provide information on the effects of phthalic anhydride exposure via the inhalation route. All of the reasonably available information identified by EPA for the inhalation route evaluated respiratory sensitization and related immune system effects and respiratory irritation. No studies investigating other endpoints following inhalation exposure to phthalic anhydride were identified by EPA. Phthalic anhydride exhibits route-specific toxicity, as also discussed in Section 4.1 and 4.2 for oral and dermal, respectively.

In the EU, phthalic anhydride is classified (GHS) as Resp. Sens. 1 (H344: May cause allergy or asthma symptoms or breathing difficulties if inhaled) (https://chem.echa.europa.eu/100.001.461/overview, accessed February 2, 2026). Additionally, OECD (2005), Health Canada (2019), Australia NICNAS (2013), and ACGIH (2025) have also identified phthalic anhydride as a respiratory sensitizer. In contrast, *o*-phthalic acid is not a respiratory sensitizer (as described in Section 4.2.2).

In the EU, phthalic anhydride is also classified (GHS) as STOT SE 3 (H335: may cause respiratory irritation) and the OECD (2005) has suggested phthalic anhydride is irritating to mucous membranes and the upper respiratory tract based on three studies in humans (Frans and Pahulycz, 1993; Baader, 1955; Menschick, 1955). Respiratory irritation differs from respiratory sensitization. Specifically, respiratory irritation is a non-immune mediated inflammatory response, and symptoms appear after a shorter duration of exposure and are generally reversible. In contrast, respiratory sensitization is an immune-mediated response that involves an initial exposure response, which is known as the induction phase, and an allergic reaction following subsequent exposure, which is known as the elicitation phase (Tarlo and Lemiere, 2014). The production of antibodies against haptenized phthalic anhydride is evidence of at least the induction of an allergic immune response to phthalic anhydride. Production of serum specific antibodies has been used as a criterion to distinguish respiratory sensitization from respiratory irritation in a recent review to establish a reference list of human chemical respiratory sensitizers (Ponder et al.,

2022). As described below, human and animal data demonstrate production of specific antibodies following exposure to phthalic anhydride.

An AOP for respiratory sensitization is currently under development (*i.e.,* AOP 39, "*Covalent Binding, Protein, leading to Increase, Allergic Respiratory Hypersensitivity Response*"), which describes the mechanistic pathway characteristic of respiratory sensitization (https://aopwiki.org/aops/39). As part of its development, the AOP includes a weight of evidence assessment for the overall AOP as a basis to consider its appropriateness for regulatory application. EPA applied AOP 39 as a framework to organize information from relevant and reasonably available human studies, animal toxicology studies, and other mechanistic data from NAMs. Appendix A.3 provides summaries of available respiratory sensitization studies considered by EPA in the current evaluation. Available data are organized by KE in the AOP for respiratory sensitization in Section 4.3.1.1. At the time of developing this draft TSD, AOP 39 was not yet endorsed by the OECD but was applied in recent publications as an organizing principle (Hargitai et al., 2024; Johnson et al., 2022; Sullivan et al., 2017), and was noted in a recent Draft Detailed Review Paper ("Draft DRP") by the OECD (*Draft Detailed Review Paper (DRP) to facilitate the Development of Test Methods to Predict the Respiratory Sensitisation Potential of Low Molecular Weight Chemicals* (OECD, 2025a)). Further, the OECD's Working Party of National Coordinators of the Test Guidelines Programme (WNT) is expected to evaluate the Draft DRP for consideration as an Integrated Approaches to Testing and Assessment (IATA) for respiratory sensitization in 2026.

EPA also considered human and animal data for the analog, trimellitic anhydride (TMA), as part of its hazard characterization and dose-response assessment (see Section 4.3.1.3 and Appendix B for EPA's read-across evaluation).

EPA's weight of scientific evidence conclusions are in Section 4.3.1.4, and its dose-response analysis is in Section 4.3.1.3. As part of its dose-response analysis, EPA developed candidate PODs for three options and then evaluated these candidate POD alongside existing inhalation hazard values from other organizations in Section 4.3.1.3.4).

4.3.1.1 AOP for Respiratory Sensitization (AOP 39)

Available data are organized by KE in AOP 39, which is shown in Figure 4-3 and summarized briefly below. KE 1 through KE 4 correspond to the induction phase of sensitization, while the adverse outcome, an allergic respiratory hypersensitivity response, corresponds to elicitation of the immune reaction upon subsequent exposure. Some KEs are shared or have overlap between the respiratory and skin sensitization AOPs, including KE 1 and KE 4. KE's 2 and 3 in AOP 39 are similar as well, but are specific to respiratory epithelial cells rather than skin epithelial cells in the skin sensitization AOP. The KEs are summarized below and in Figure 4-3, and described more thoroughly thereafter. EPA highlights, where relevant, mechanistic alignment between the KEs for AOP 39 and that of dermal sensitization described above in Section 4.2.1.1.

- **KE 1: Covalent binding to proteins (OECD TG 442C):** KE 1, the MIE, involves covalent binding of an electrophilic chemical to cysteine or lysine residues in proteins. Covalent binding forms a hapten complex, which can be recognized by immune cells and trigger subsequent KEs in the AOP leading to sensitization. This KE is shared with KE 1 in the skin sensitization AOP (OECD, 2014)
- **KE 2: Increased secretion of pro-inflammatory mediators:** The hapten complex may react with cell surface proteins on respiratory epithelial cells to activate inflammatory cytokines and induce cytoprotective gene pathways. This is a cellular response.

- **KE 3: DC activation and maturation:** The hapten complex can alternatively directly act on DCs to induce the production of inflammatory cytokines and cell surface molecules, as well as stimulate the mobilization of DCs. This is a cellular response and is similar to KE 3 in the AOP for skin sensitization, but involves crosstalk of DCs in the lung and airway epithelial cells.
- **KE4: T tell activation, proliferation, and differentiation:** Activated, mature DCs present processed antigen (*i.e.,* the chemical-hapten-complex) via MHC receptors to the T-cell receptor on T-cells in the local lymph nodes near the site of contact. Antigen presentation results in activation and proliferation of T-cells in the lymph node. This is an organ-level response. This KE is similar to KE 4 in the of Skin Sensitization AOP (OECD, 2014), but may elicit a distinct cytokine profile specific to respiratory sensitizers.
- **Adverse Outcome: sensitization of the respiratory tract:** The adverse outcome is immunological priming and sensitization of the respiratory tract, which represents a shift in disease status. The adverse outcome reflects the first of a two-step process in the development of chemical respiratory allergy, defined by Sullivan et al. (Sullivan et al., 2017) as "an immune-mediated hypersensitivity reaction to an exogenous low-molecular weight chemical resulting in symptoms such as asthma and rhinitis." The second step reflects a subsequent exposure to the respiratory tract to the same chemical, resulting in elicitation of symptoms.

Evidence for each KE is summarized below in Section 4.3.1.1.1 through Section 4.3.1.1.4.

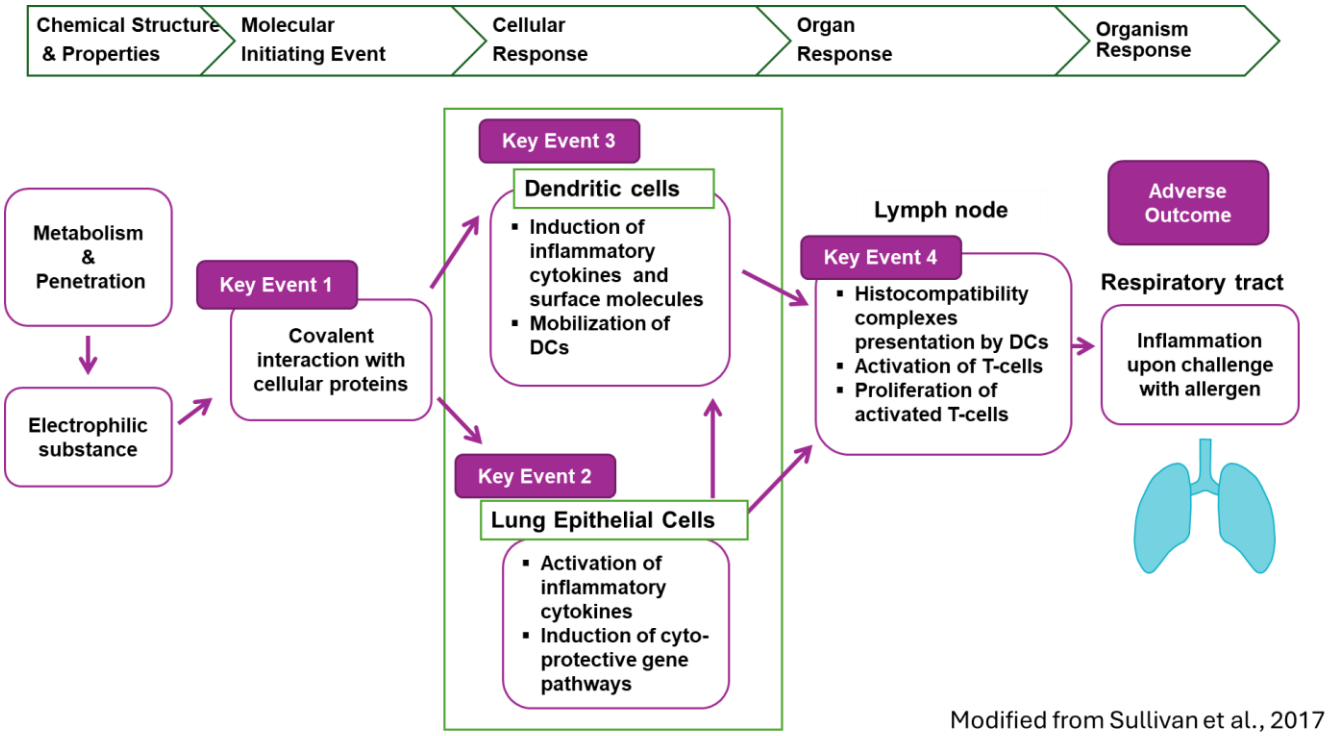

Figure 4-3. AOP for Covalent Binding, Protein, Leading to Increase, Allergic Respiratory Hypersensitivity Response and Common Assays to Evaluate KEs
Adapted from https://aopwiki.org/aops/39 and modified from (Sullivan et al., 2017).

4.3.1.1.1 KE 1: Covalent Binding to Proteins

AOP 39 shares the same MIE as the AOP for dermal sensitization (Section 4.2.1.1) – covalent binding of an electrophilic chemical to cysteine or lysine residues in proteins (Hargitai et al., 2024; Kimber et al., 2018; Sullivan et al., 2017). At the molecular level, covalent binding of a low-molecular weight

sensitizer with cysteine or lysine residues forms a hapten complex, which can be recognized by immune cells and trigger subsequent KEs in the AOP and lead to sensitization.

Available evidence for phthalic anhydride relevant to KE 1 includes the aforementioned peptide-depletion assay, the DPRA and kDRPA. As discussed in Section 4.2.1.1.1, phthalic anhydride has been evaluated twice in the DPRA and once in the kDPRA, giving a positive result in all three assays (Table 4-6). As described above in Section 4.2.1.1.1, phthalic anhydride is believed to preferentially react with lysine residues via acylation (Johnson et al., 2022; Krutz et al., 2021; Wareing et al., 2017; Dik et al., 2016; Piroird et al., 2015; Bauch et al., 2012; Enoch et al., 2009). The preferential association of phthalic anhydride with lysine is supported by the available data, which indicate higher percent depletion of lysine residues compared to cysteine restudies (Table 4-6).

4.3.1.1.2 KE 2: Increased Secretion of Pro-inflammatory Mediators

The first KE after the haptenization is increased inflammation, which is reflected by increased secretion of pro-inflammatory mediators in the respiratory tract. The hapten complex can react with cell surface proteins and/or endogenous proteins such as pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) and/or damage associated molecular patterns (DAMPS) to activate response pathways. The response pathways involved in KE 2 in AOP 39 are similar to those induced in KE 2 the skin sensitization AOP (Section 4.2.1.1), the induction of cytoprotective cellular pathways (*e.g.,* the Nrf2-Keap1-ARE regulatory pathway). PRR recognition of PAMPs/DAMPs also triggers the release of pro-inflammatory cytokines and chemokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which are also called alarmins. The production of inflammatory cytokines and chemokine's attracts DCs, which express cytokine receptors such as TSLP receptor (TSLPR), IL-17RA (interleukin-17 receptor A, and IL1RL1 (interleukin-1 receptor-like-1). As discussed further down, DCs activated by alarmins promote a Th2 inflammatory response and favors production of cytokines IL-4, IL-5, and IL-13 (KE 3), and subsequently a Th2 response (KE 4) (Hargitai et al., 2024).

EPA evaluated the evidence for KE 2 across reasonably available studies and identified some evidence relevant for KE 2 in one *in vitro* study performed in a co-culture model of the respiratory tract. No *in vivo* studies were identified that provided data for KE 2. The results of these KE 2-related assays are provided in Table 4-14.

The ALIsens assay is a three-dimensional co-culture *in vitro* model representing the alveolar barrier which combines three human cell lines. As part of the development of the ALIsens assay (an *in vitro* system for detection of respiratory sensitizers), Chary et al. (2019) co-cultured alveolar type II epithelial cells (A549), endothelial cells (EA.hy926), macrophage-like cells (PMA-differentiated THP-1), and dendritic-like cells (non-differentiated THP-1) at an air-liquid interface to recapitulate the alveolar barrier, and exposed cells to aerosolized phthalic anhydride for 48 hours. After 48 hours, THP-1 cell viability was measured and the CV75 (concentration resulting in 75% cell viability) was determined to be 148 $\mu g/cm^2$. THP-1 cells were then exposed to the CV75 for 24 hours. Phthalic anhydride was shown to increase secretion of several cytokines, including of the cytokines C-C motif chemokine ligand 20CCL20 (CCL20; 2.5-fold increase), IL-10 (2-fold); and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF; 2.4-fold increase). There is evidence from animal and human studies that GM-CSF is linked to inflammation in the airways. GM-CSF is released by epithelial cells during allergic lung inflammation and has been implicated in allergic airway inflammation in mouse models (Asquith et al., 2008; Su et al., 2008) and elevated GM-CSF levels have been observed in found in asthma patients (Saha et al., 2009). IL-10 is generally recognized as an anti-inflammatory cytokine that suppresses the activity of many innate and adaptive immune cells, however, there is incomplete understanding of how

to interpret its role in allergic airway inflammation (Hendriks, 2023). During sensitization, IL-10 promotes DC recruitment (KE 3) by enhancing the release of CCL20 by lung epithelial cells (KE 3) (Hendriks, 2023). Because there is not yet a data interpretation procedure for this KE, it is not yet clear how to interpret the observed increase in GM-CSF and CCL20, which would be consistent with increases in inflammatory cytokines, and with the increase in IL-10, which is not. It is also unclear how to interpret the significance of the magnitude of the increases in these cytokines, because thresholds or definitive cut-off levels are not yet available for any of the cytokines or chemokines analyzed that allow clear indication of a positive or negative result (Gutleb et al., 2026). Therefore, EPA considered the data to be inconclusive for KE 2.

Additional data from Chary et al. (2019) relevant to KE 3 is discussed below (*e.g.,* TSLPR upregulation).

Table 4-14. Summary of *In Vitro* Data for KE 2 in Respiratory Sensitization AOP

Cell Type	Result	Remarks	Reference
ALIsens	Inconclusive [a]	- ↑ GM-CSF(2.4-fold), ↑ IL-10 (2-fold); ↑ CCL20 (2.5-fold) [b] - Cytokine release from epithelial cells	(Chary et al., 2019)

AOP = adverse outcome pathway; GM-CSF = granulocyte-macrophage colony-stimulating factor
[a] No definitive cut-off levels currently exist for any of the cytokines or chemokines analyzed for KE 2 that allow clear differentiation between sensitizers and non-sensitizers, but emerging evidence suggests that the overall pattern of changes across multiple cytokines is more reliable basis for future classification.
[b] Fold change relative to vehicle control.

4.3.1.1.3 KE 3: Dendritic Cell Activation and Maturation

KE 3 reflects activation of dendritic cells (DCs). DCs become activated and monocyte-derived DCs are recruited from the blood in response to signals from inflammatory mediators from KE 2. Alternatively, chemical-hapten conjugates can directly activate naïve DCs. The AOP depicts two routes to KE3: KE1 branches to KE2 or proceeds directly to KE3 Figure 4-3 (Hargitai et al., 2024). DC activation results in a mature DC that has a new phenotype and function. Mature DCs engulf, process, and migrate to local lymph nodes to present the processed hapten to the naïve T-cell receptor (TCR) via the MHCII molecule. The genetic region encoding MHC class II is one of the most polymorphic gene clusters, which likely contributes to interindividual differences in allergic responses (Yuhki and O'Brien, 1990), including that of respiratory sensitization, which is discussed as a PESS in Section 7.2. Mature DCs express co-stimulatory ligands CD54, CD86, CD80, and other molecules such as OX40 Ligand (OX40L) and TSLPR. Binding of TSLP cytokine to the TSLPR of a DC contributes to Th2 response.

KE 3 can be evaluated using the GARDair assay and the ALIsens assay. The GARDair assay is an immune cell assay that is based on the use of a human DC line, SenzaCell™ and employs genomics and machine leaning for prediction in a process analogous to that of GARDskin described above in Section (4.2.1.1.3). A positive result in the assay reflects activation of signaling through IL-7 receptor (IL7R) and cytokine receptor-like factor 2 (CRLF2), which encode proteins that form TSLPR. Binding of the TSLPR drives Th2 differentiation (Hargitai et al., 2024; Kimber et al., 2014 2014, 2542234; Paul and Zhu, 2010). The ALIsens assay is a three-dimensional co-culture *in vitro* model representing the alveolar barrier which combines three human cell lines: alveolar type II epithelial cells (A549), endothelial cells (EA.hy 926) and monocytes (THP-1). The ALIsens assay evaluates the chemical's respiratory sensitization potential at an air-liquid interface and uses cytokine profiles and cell surface markers as a readout (Chary et al., 2019).

Phthalic anhydride has been evaluated in two *in vitro* assays developed to detect respiratory sensitizers, including the GARDair assay once and the ALIsens assay twice (NTP, 2026; Invitrolize, 2024; Chary et al., 2019). The results of these assays are provided in Table 4-15.

In the initial ALIsens assay, phthalic anhydride was included as part of the training set for the development of the test system (Chary et al., 2019). Briefly, Chary et al. exposed cells to phthalic anhydride for 48 hours, measured cell viability, and determined the CV75 (concentration resulting in 75% cell viability) to be 148 µg/cm^2. Cells were then exposed to the CV75 for 24 hours, and then CD54 and CD86 were measured on the surface of dendritic-like THP-1 cells. Phthalic anhydride induced a 186% increase in CD54 expression, while CD86 was upregulated 122%. Additionally, exposure to phthalic anhydride was shown to upregulate TSLPR 162% (a cytokine involved in DC activation); increase secretion of the cytokines CCL20 (2.5-fold increase); and modulate mRNA expression of IL1RL1 (1.2-fold increase) and Class II MHC transactivator (CIITA; 1.3-fold decrease). The authors do not provide sufficient information to indicate positive or negative result, and there is no data interpretation procedure in that study for EPA to use as a guide. However, in the context of the subsequent 2024 study described below (Invitrolize, 2024), the study results in Chary et al. (2019) would be considered negative because the increases in CD86 expression and CD54 expression are below the thresholds that indicate a positive result.

In the second ALIsens assay, cells were exposed to the CV75 concentrations of phthalic anhydride (198 µg/cm^2) for 24 hours, and then CD54 and CD86 were measured on the surface of dendritic-like THP-1 cells (Invitrolize, 2024). Three separate replicates of the experiment were conducted for each chemical. A positive result was indicated by exceeding a 150% threshold for CD86 or 200% for CD54 for at least one of these cell surface markers, which is consistent with h-CLAT assay principles (OECD, 2023b). The positive control (LPS+TSLPR) gave anticipated results (*i.e.,* 35- and 50-fold increases in CD86 and CD54). Similar to the first study by Chary et al. (2019), phthalic anhydride induced CD86 cell surface marker above the threshold of 150%, to an average value of 170% (values across 3 experiments: 97%, 242%, 172%), but did not induce the average (*i.e.,* 155%) expression of CD54 cell surface marker over the 200% threshold (values across 3 experiments: 93%, 125%, 248%). Under the conditions of the assay, phthalic anhydride was considered positive for respiratory sensitization.

EPA collaborated with NICEATM and Inotiv to nominate phthalic anhydride for testing by NICEATM in the GARDair assay. The nomination was accepted, and NICEATM initiated testing on the potential for phthalic anhydride to cause respiratory sensitization using the GARDair assay (NTP, 2026). The GARDair assay evaluates DC activation in a human myeloid leukemia cell line MUTZ-3, SenzaCell, as a DC surrogate. GARDair assays were performed according to the SOPs provided by SenzaGen (method developer). Briefly, NICEATM first conducted a dose-range finding study by exposing cells to phthalic anhydride for 24 hours and measuring cell viability. The dose range finding study determined that the test concentration for sensitization testing would be 500 µM, which is within the cytotoxicity acceptance range (*i.e.,* 84.5−95.4%). Cells were then exposed to 500 µM phthalic anhydride in 0.2% DMSO or 0.2% DMSO (solvent control) for 24 hours and harvested for RNA extraction for transcriptomic analysis. The readout of the assay is a transcriptional quantification of the genomic predictors using Nanostring nCounter technology, whereby chemicals are predicted as either Sensitizers or Non-sensitizers by a Support Vector Machine (SVM) Model (*i.e.,* a machine learning method that is trained using data collected from cell stimulations with known chemicals). The endpoint value of each GARD measurement is a derived decision value (DV) from the SVM Model. Mean DV greater than zero result in classification of "Sensitizer." The mean DV for phthalic anhydride was 4.96; therefore, phthalic anhydride was considered positive for respiratory sensitization.

Overall, the available data from two of three *in vitro* assays support the conclusion that phthalic anhydride is a respiratory sensitizer and provide data for KE 3.

Table 4-15. Summary of Data for KE 3 in Respiratory Sensitization AOP

Assay	Result	Remarks	Reference
ALIsens	Negative[a]	- Gene expression changes in THP-1 cells following exposure to CV75 of 148 µg/cm^2 - ↑ CD86 expression (122%); ↑ CD54 expression (186%); ↑ TSLPR expression (162%); No change in OX40L	(Chary et al., 2019)
ALIsens	Positive	- Cells exposed to 148 µg/cm2 (CV75 determined by Chary et al. (2019) - Above 1.5-fold threshold for CD86 expression (avg. of 170% across 3 experiments) - Below 2-fold threshold for CD54 expression (avg. of 155% across three experiments)	(Invitrolize, 2024)
GARDair	Positive[b]	- Mean decision value (DV) = 4.96 (DV > 0 indicates a positive result) - No cytotoxicity at test concentration (500 µM)	(NTP, 2026)

CV_{75} = the concentration at which 75% cell viability occurs; DV = decision value; GARD = Genomic Allergen Rapid Detection

[a] In the original study, authors do not provide sufficient information to indicate positive or negative result. However, in a follow-up study by the same research group (Invitrolize, 2024), thresholds for determining a positive or negative result are established (*i.e.,* positive result indicated by >200% increase in CD54 or >150% increase in CD86). Under the criteria outlined by Invitrolize (2024), the results reported by Chary et al. would be considered negative.

[b] The readout of the assay is a transcriptional quantification of the genomic predictors using Nanostring nCounter technology. Chemicals are predicted as either Sensitizers or Non-sensitizers by a Support Vector Machine (SVM) Model (*i.e.,* a machine-learning method that is trained using data collected from cell stimulations with known chemicals). The endpoint value of each GARD measurement is a derived decision value (DV) from the SVM model. Mean DV greater than zero result in classification of "Sensitizer."

4.3.1.1.4 KE 4: T-Cell Activation, Proliferation, and Differentiation

Mature, Th2-skewed DCs migrate to draining lymph nodes, where they interact with the T-cell receptor (TCR) of T-cells, leading to T-cell activation, proliferation, and differentiation into effector helper T-cells and memory populations (Hargitai et al., 2024; Kimber et al., 2018) (KE 4). It is thought that respiratory sensitizers favor a Th2 type response, where the Th2 effector cells are predominantly involved in the elicited immune response (Hargitai et al., 2024). Th2 polarization is reflected by the cytokine profile in the cellular milieu during/after T-cell activation (Hargitai et al., 2024). The cytokine profile expected for a respiratory sensitizer includes IL-4 and IL-10 (De Jong et al., 2009; Dearman et al., 2003). However, the mechanism for T-cell activation following exposure to respiratory sensitizers is not fully understood.

T-cell activation and proliferation in AOP 39 (*i.e.,* KE 4) can be evaluated using the LLNA with subsequent cytokine fingerprinting (Hargitai et al., 2024). The standard LLNA (OECD TG 429) cannot be used to identify respiratory sensitizers that are also skin sensitizers because the assay cannot differentiate between lymphocyte proliferation induced by skin versus respiratory sensitizers. However, in analogy to the standard dermal LLNA, a respiratory LLNA was developed by Arts et al. (2008), which EPA considered in this Section of its hazard assessment. Additionally, it is thought that respiratory and skin sensitizers induce different immune responses that can be determined via cytokine fingerprinting. Therefore, in addition to data from the respiratory LLNA, EPA evaluated data on cytokine expression (in lymph nodes following T-cell activation by the mature DCs) and helper T-cell

phenotype (*i.e.,* Th2, Th1, *etc*.) as part of the evidence for KE 4. EPA identified one *in vivo* study in mice exposed to phthalic anhydride via inhalation that provide relevant data for KE 4.

In Vivo Evidence

Arts et al. (2008) developed a "respiratory LLNA" where male BALB/c mice were exposed via nose-only inhalation to 15 mg/m^3 phthalic anhydride (measured concentration: 14.2 ± 1.5 mg/m^3; MMAD [mass median aerodynamic diameter] = 2.6 ± 4.4 µm) for 45, 90, 180, or 360 minutes per day for 3 consecutive days. Vehicle controls were exposed to acetone for 360 minutes for 3 days. The test methods were similar to the standard LLNA (OECD, 2010). In analogy to the standard skin LLNA, the authors considered a positive result an SI of 3 or more in the respiratory tract draining lymph nodes. The authors reported that the response was positive but noted that it was variable because the response was inconsistent across exposure durations (*i.e.,* there was no duration-response relationship). These data are summarized in Table 4-16. EPA considered these data positive for KE 4 because 2 of 6 animals had an SI of at least 3 in the 360-minute exposure group for phthalic anhydride compared to 0 of 11 animals in the 360-minute exposure group for vehicle controls.

Table 4-16. Proliferation Data in the Mandibular Lymph Nodes Following Inhalation Exposure to 14.2 ± 1.5 mg/m^3 Phthalic Anhydride

Duration (minutes)	Mean ± SEM [^{3}H]-TdR Incorporation (cpm)	SI (Mean ± SEM) [a]	No. Animals Positive/ Total No. Animals[b]
45	4,238 ± 843	2.9 ± 0.57	3/6
90	5,352 ± 801	3.6 ± 0.54	4/5
180	3,330 ± 223	2.3 ± 0.15	0/5
360	4,289 ± 760	2.9 ± 0.52	2/6
360[c]	1,471 ± 151	1.0 ± 0.10	0/11

CPM = counts per minute; SEM = standard error of measurement; SI = stimulation index
[a] Data shown in Figure 1 in Arts et al. (2008) and Table 1 in De Jong et al. (2009)
[b] A positive response is indicated by an SI ≥ 3
[c] Vehicle controls exposed to acetone for 360 minutes.

Arts et al. (2008) examined cytokine profiles in addition to the modified "respiratory LLNA," and published these data in a separate publication by De Jong et al. (2009). De Jong et al. (2009) isolated the mandibular and auricular lymph node cells to determine the cellular proliferation and cytokine production following *ex vivo* stimulation with Conclavin A. The authors reported increases in IL-4 and IL-10 at the 45- and 90-minute timepoints, with no change at other timepoints. However, because the comparator was exposed to acetone vehicle for 360 minutes, the only valid comparison group is the 360-minute group, which did not exhibit any change in any cytokine measurement.

4.3.1.1.5 Adverse Outcome: Allergic Respiratory Hypersensitivity Response

The adverse outcome is sensitization of the respiratory tract, which occurs in two phases. The first phase, induction, occurs following an individuals' first exposure and involves development of an allergic response that is reflected by the upstream KEs and manifests as immunological priming and a change in disease state (*i.e.,* sensitized). The second phase occurs when a sensitized individual is exposed again to the same substance and clinical symptoms develop an allergic hypersensitivity reaction (Sullivan et al., 2017). The adverse outcome reflects the allergic hypersensitivity response that occurs during the elicitation phase. EPA identified relevant information from humans and animals for the

adverse outcome, which collectively provide data consistent with the adverse outcome of allergic respiratory hypersensitivity response to phthalic anhydride, as described below. Evidence integration of human, animal and mechanistic streams of evidence is in the next section (Section 4.3.1.2).

Human Evidence

There is human evidence for the adverse outcome from epidemiological studies as described below. In humans, the hypersensitivity reaction can be evaluated by evidence of an immune response with clinical outcomes such as rhinitis, rhino-conjunctivitis, and asthma, as well as respiratory allergy symptoms such as nasal congestion, cough, wheezing, chest tightness, airflow obstruction, and bronchoconstriction. Immune responses specific to phthalic anhydride can be determined specific IgG or IgE detection (Hargitai et al., 2024).

EPA identified five epidemiological studies that evaluated the association between inhalation exposure to phthalic anhydride and various respiratory effects (Barker et al., 1998; Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986; TOMA, 1982, 1981, 1979). Three of the available epidemiological studies demonstrate that phthalic anhydride is a respiratory sensitizer which is consistent with the conclusions of other existing assessments. Available studies are summarized in Table 4-17 and briefly discussed below. The evidence is discussed in detail in the current section as evidence for the adverse outcome in AOP 39.

Nielsen et al. (1988) is a low-to-medium-quality, cross-sectional study of workers with concurrent exposure monitoring and immunologic testing that evaluated symptoms in 60 workers exposed to phthalic anhydride at two plants in Sweden that manufactured alkyd and unsaturated polyester resins. Workers were classified as "heavily" (n = 28 reactor loaders; n = 7 repair men) and "slightly" (n = 25) exposed based on their job. Heavily exposed workers had been employed for an average of 13 years (range: 0–43 years), while slightly exposed workers had been employed for an average of 12 years (range: 0.3–40 years). A reference group of 22 male workers from a food-processing factory was also evaluated. Heavily exposed workers were exposed to phthalic anhydride dust for 5 to 30 minutes, 1 to 2 times during a work shift when emptying 25 kg bags of flaked phthalic anhydride into a reactor, with respirator use reported to be irregular. Measurable phthalic anhydride air concentrations (0.1 to 0.2 mg/m^3 or 1.5 to 3% of the initial concentration) were observed close to the reactors for up to 60 minutes. Other known sensitizers (*i.e.,* maleic anhydride, isophthalic anhydride, TMA) were also reported to be used at the facilities, albeit at a lower amount than phthalic anhydride.

Workers were reported to have "mostly" used respirators when working with maleic anhydride and isophthalic anhydride, and to have "always" used respirators when handling TMA. The mean peak air concentration of phthalic anhydride during reactor loading was 6.6 mg/m^3 (range: 1.5–17.4 mg/m^3), while the 8-hour TWA was 0.4 mg/m^3. Serum antibodies were measured in groups of workers and compared to that of 22 workers from the reference group. Exposed workers presented with conjunctivitis (high exposure: 16/35 [46%]; low exposure: 5/25 [25%]; control: 0/22); rhinitis (high exposure: 14/35 [40%]; low exposure: 5/25 [20%]; control: 0/22); rhinoconjunctivitis (high exposure: 6/35 [17%]; low exposure: 3/25 [12%]; control: 0/22); chronic bronchitis (high exposure: 6/35 [17%]; low exposure: 1/25 [4%]; control: 0/22); and asthma (high exposure: 5/35 [14%]; low exposure: 0/25; control: 0/22). Of the five workers presenting with asthma, three presented with dual type asthma with both immediate and late onset reactions, while two presented with late type asthmatic responses. Serum levels of specific IgG against phthalic anhydride-HSA were significantly increased in subjects of the high exposure group. Furthermore, compared to the asymptomatic patients, the asthmatic subjects' specific IgG levels were significantly greater. One of five workers with asthma had a positive skin prick test against phthalic anhydride conjugated with human serum albumin. Serum levels of other antibodies, including total and

phthalic-anhydride-specific IgE, IgM, and IgA, did not vary across exposures groups; however, one of the subjects with conjunctivitis, rhinitis, and asthma had increased level of specific IgE.

EPA identified limitations that contributed uncertainty in the utility of Nielsen et al. (1988) for dose-response. Due to understandable practical challenges, the study did not distinguish between sensitized and newly sensitized workers, obscuring interpretation of exposure–response by mixing induction and elicitation phases of respiratory sensitization. Additionally, the study had a small sample size and only assessed current workers, potentially introducing a healthy worker effect and consequently reducing observed symptom prevalence. However, this would introduce a bias toward the null. Additionally, there was a limited number of short-term personal air samples. The exposure was characterized by job task (heavy vs. low) with limited short-term measurements and group averaged values, rather than continuous, individual level peak monitoring or long-term personal histories. There was also co-exposure to maleic anhydride, isophthalic anhydride, and TMA at the plant. Respirator use was reportedly used, but the usage was irregular. Overall, the study had several limitations given its cross-sectional design, limited exposure characterization, small case numbers, and co-exposures that were unaccounted for (*i.e.,* through statistical models, etc.). Because some factors likely bias toward the null (*e.g.,* healthy worker effects and nondifferential exposure misclassification) while others may bias away from the null (*e.g.,* confounding by co-exposures such as TMA), the net direction of bias is uncertain. EPA's confidence is supported by the study's exposure contrast and complementary immunologic evidence, which the Agency considered a strength compared to other available studies in humans described below.

Nielsen et al. (1991), a low-quality, cross-sectional comparison of currently employed workers with phthalic anhydride exposure versus unexposed controls, evaluated symptoms in 23 male workers exposed to phthalic anhydride in two plants producing alkyd and/or unsaturated polyester resins. Nineteen of those workers, who regularly worked on loading of the reactors had high levels of exposure to phthalic anhydride. The other four had considerable exposure to phthalic anhydride, however, they did not regularly work as loaders. The median duration of exposure for the whole group of 23 workers was approximately 12 years (range: 1–35 years). Of the 23 workers, 8 had low-exposure jobs by the time of the study. A total of 19 men were classified as highly exposed to phthalic anhydride (median exposure of 7 years; range: 0–33 years). Heavily exposed workers were exposed to phthalic anhydride dust for 5 to 30 minutes, 0 to 3 times during a work shift when emptying 25 kg bags of flaked phthalic anhydride into a reactor; respirator use was reported to be irregular. Other known sensitizers (*i.e.,* maleic anhydride, isophthalic anhydride, and TMA) were also reported to be used at the facilities, albeit at lower amounts than phthalic anhydride.

Personal respiratory protective devices were reported to be used only irregularly for tasks that resulted in inhalation to phthalic anhydride. Workers were reported to have "mostly" used respirators when working with maleic anhydride and isophthalic anhydride, and to have "always" used respirators when handling TMA. The air levels of the 23 workers exposed to phthalic anhydride ranged from 1.5 to 17 mg/m^3 (mean: 6.6 mg/m^3). Compared to 18 control subjects, exposed workers presented with conjunctivitis (48% vs. 6%), rhinitis (39% vs. 0%), rhino-conjunctivitis (22% vs. 0%), and dry cough (17% vs. 0%). Work-related asthma was seen in two of the exposed workers. The type of asthmatic reactions (*i.e.,* immediate, late, or dual type) was not reported for these workers. While there was no difference in the readings for specific IgE against phthalic anhydride, the exposed workers had significantly higher levels of total serum IgE (medians, 32 vs. 15 KIU/1) and specific-IgG against phthalic anhydride-HSA (ELISA ratios 0.31 vs. 0.12). Vital capacity forced expiratory volume (FEV1), and closing volume as a percentage of vital capacity did not indicate any significant variations in lung function between exposure groups. There were no subclinical lung effects from phthalic anhydride

exposure. The study had some uncertainties that led the EPA to question its appropriateness for dose-response. It also had limited task-based sampling and no individual-level long-term or peak exposure metrics and there was irregular respirator use that was not accounted for in the dose. Other anhydrides such as maleic anhydride, isophthalic anhydride, and TMA were present in the plants and may confound the reported associations. Given the study's cross-sectional sample size, co-exposure with other anhydrides, limited exposure characterization (exposed vs. 1 control), and potential confounding, the data are not suitable for quantitative dose-response analysis.

Wernfors et al. (1986) is a low-quality cross-sectional study of current workers plus a retrospective survey of former workers that evaluated 118 current and former workers exposed to phthalic anhydride dust for at least two months in four plants producing alkyd or polyunsaturated polyester resins. Workers were exposed to phthalic anhydride dust for 10 to 30 minutes, several times a day during a work shift when emptying 25 kg bags of flaked phthalic anhydride into a chemical reactor, with respirator use reported to be irregular. Other known sensitizers such as maleic anhydride were used in all plants albeit at a lower amount than phthalic anhydride, while TMA was used once a month in one plant. Workers were reported to have 'mostly' used respirators when working with maleic anhydride, and to have "always" used respirators when handling TMA. During various direct phthalic anhydride handling operations (*i.e.,* loading of reactors, handling of empty bags, assisting loader), the TWA breathing zone phthalic anhydride levels at two factories ranged from 3 to 13 mg/m^3, but for other types of labor (*e.g.,* cleaning, reactor sampling), they were less than 0.3 mg/m^3. Of this cohort of 118 phthalic anhydride exposed current and former workers, 13 (11%) suffered from chronic productive bronchitis, 28 (24%) from work-related rhinitis, and 21 (18%) from work-associated asthma. Of the 21 workers suffering from work-related asthma, 4, 9, and 6 presented with immediate, late, and dual type asthmatic reactions, while the reaction type could not be determined in 2 workers. Eleven of the 21 workers suffering from asthma were skin scratch tested with pulverized phthalic anhydride. Of these 11 workers, 3 (27%) had phthalic anhydride positive skin tests. Two subjects with positive skin prick tests volunteered for a specific inhalation challenge (SIC), short-term (5 to 10 minute) exposure to 0.5 and 6 mg/m^3 phthalic anhydride, respectively, which resulted in positive bronchial provocation.

The study had some uncertainties that led the EPA to question its appropriateness for dose-response. There is a discrepancy in asthma prevalence in the abstract (28%) vs. results (21/118 = 18%). Former workers had significantly higher bronchitis prevalence and non-significant higher rhinitis/asthma which may suggest survivor bias for some outcomes. The authors did not present individual-level cumulative or peak exposure metrics and there was irregular respirator use not integrated into dose estimate. There was insufficient graded exposure for modeling concentration-response. Given the design limitations (cross-sectional study), limited exposure characterization, and potential survivor and reporting biases, the study is not suitable for quantitative dose-response analysis.

Barker et al. (1998), a medium-quality study, identified a cohort of 506 workers, in the United Kingdom exposed to acid anhydrides at four industrial factories. Of the 506 workers in the target population, 401 were reached and completed all necessary surveys to participate in the study. Besides phthalic anhydride, other known sensitizers (*i.e.,* maleic anhydride, TMA) were reported to be used at the factories. Factories 1, 3, and 4 mostly used phthalic anhydride in the production of alkyd resins, although TMA and maleic anhydride were also used, while factory 2 only use TMA. Workers who manually loaded TMA in factories 1, 3, and 4 reported to have "almost always" worn full respirators and disposable suits and air feed hoods when working with the chemical; however, respiratory protection was "almost never" used when loading phthalic anhydride. Each factory's employees were categorized by job title. Following a random selection process within job titles, employees were instructed to wear an air sampling pump for the duration of a full work-shift and while doing specific tasks.

Over a period of 2 to 4 weeks, a sample of around 1 out of 10 complete shifts completed in each job title was taken at each factory. Initially, cutoff criteria for exposure groups were set at 10 and 100 µg/m^3 without considering the health outcomes. Past exposure to phthalic anhydride in the factories ranged from 0.4 to 2,500 µg/m^3 in factory 1, 2.2 to 140 µg/m^3 in factory 3, and 1.5 to 60 to µg/m^3 in factory 4. The current full shift exposure measurements reported for phthalic anhydride was 2.2 µg/m^3 in factory 1, 5.5 µg/m^3 in factory 3, and 4.5 µg/m^3 in factory 4. Besides answering self-administered questionnaire on health, smoking habits and work experience, participants also had a skin prick test. The survey found that workers with acid anhydride sensitization were older than those without acid anhydride sensitization, and male workers were the only ones who reported respiratory symptoms related to their jobs or who were sensitized. Compared to current employees, those who had left the area of acid anhydride exposure were more likely to experience work related respiratory symptoms and sensitization. Respiratory symptoms related to the workplace acid anhydride exposure were more common among workers who were sensitized to acid anhydrides. New self-reported work-related respiratory symptoms (defined as chest tightness, difficulty breathing, or wheezy or whistle sound coming from chest) were reported by 34 workers (8.8%), including 28 former and 6 current workers (6, 13, 5, and 10 from factories 1, 2, 3, and 4, respectively), while 12 workers (3.2%), including 11 former and 1 current (0, 8, 1, and 3 from factories 1, 2, 3, and 4, respectively), had a positive immediate skin prick reaction test to acid anhydride-HSA conjugate. Workers with positive skin prick tests were more likely to have work-related respiratory symptoms (10 of 12 workers with positive skin prick tests also reported respiratory symptoms).

There was an observed statistically significant increase in the odds (odds ratio=3.40, 95% CI: 1.29–8.96) of new work-related respiratory symptoms from all acid anhydride factories in workers in the medium exposure group (10 to <100 µg/m^3) compared with the lowest exposure level (<10 µg/m^3), however, the odds ratio (OR = 5.58, 95% CI: 0.89–35.21) of new work-related respiratory symptoms was not statistically significant in the high exposure group (>100 µg/m^3). The study had some uncertainties that led the EPA to question its appropriateness for dose-response. Other anhydrides such as maleic anhydride and TMA were present in the plants and there were no phthalic anhydride specific outcomes. Given that this study does not provide phthalic anhydride specific data, makes the study unsuitable for dose-response analysis.

A series of low-quality, cross-sectional occupational health studies by Tabershaw Occupational Medicine Associates, P.A. (TOMA) (TOMA, 1982, 1981, 1979) were performed on employees of the Kippers' Bridgeville, Pennsylvania plant that produced phthalic anhydride, polyester resin and some alkyd resins for chemical processors, paint and coatings. Other known chemicals (*i.e.,* maleic anhydride, amino resins and alkyd resins) were also reported to be used at the plant. The lung, liver, kidney, blood, and skin were the targets of a general screening profile because of the high number of chemicals produced at the plant with non-specific health consequences. In the 1982 TOMA study (TOMA, 1982), an x-ray of the chest revealed nine cases of suspected mild pneumoconiosis. Additionally, pleural thickening was present in four of these individuals. Three more had thickening of the pleura but no other accompanying problems. The authors indicated that there is no evidence that any job exposures occurred at this plant that might have led to the x-ray abnormalities that were found. The workers did not exhibit any additional anomalies in higher proportions than the general population of the same age groups (TOMA, 1982, 1981, 1979). One worker, a 64-year-old cigarette smoker, was found to have lung cancer, the authors indicated that it was unrelated to work exposure. While several workers exhibited anomalies, these were usually small and unrelated to their jobs. The authors noted that there were no significant abnormalities in the TOMA studies that would suggest harmful effects on the skin, kidney, or liver. There were no reported cases of occupational asthma and other outcomes that may be related to respiratory sensitization were not evaluated (*e.g.,* serum antibodies against phthalic anhydride antigens).

Taken together, the TOMA studies did not identify any illnesses or anomalies that may be directly linked to occupational exposures to phthalic anhydride. The studies had some uncertainties that led the EPA to question its appropriateness for dose-response. There was no clear association between exposure to phthalic anhydride and increased rates of allergic sensitization or occupational asthma in these study screenings. The principal occupational findings centered on dermatologic effects (folliculitis/chloracne) consistent with coal tar exposures. The study is a cross-sectional snapshot among current workers with variable participation. There was no quantitative phthalic anhydride measurements reported, and no individual-level exposure characterization. The studies did not report any specific phthalic anhydride exposure measurements or quantitative job-exposure matrix and no resolution of peak exposures that are relevant for sensitization. There were also no phthalic anhydride specific health outcomes, the outcomes are non-specific and there is no incident follow-up. The plant had other anhydrides such as maleic anhydride and variable respirator use further obscure the dose attributed to phthalic anhydride. Across the TOMA's studies, there was no clear excess of allergic sensitization or asthma specifically attributable to phthalic anhydride. Allergic markers (eosinophils, total IgE) were elevated in some workers but were distributed across plant areas and not consistently linked to symptoms or function. However, the studies were not designed to detect phthalic anhydride -specific sensitization or to quantify exposure-response as they lacked phthalic anhydride exposure measurements, phthalic anhydride-specific immunologic testing, and incident case follow-up, and were subject to selection and confounding. Consequently, these studies are not suitable for quantitative dose-response analysis for phthalic anhydride.

EPA also received one submission via Section 8(e) from Carbide and Carbon Chemicals Corporation (1992) which described evidence for human sensitization responses and asthma in workers exposed to phthalic anhydride. The report includes a memorandum from 1962 of a phthalic anhydride production plant employing 20 to 25 men who had been working six months at the time of the report. Of these, the report indicates that, "five cases of asthmatic bronchitis have developed among men working in the unit and one of these has developed a clinical picture of sensitization in that he develops symptoms of asthma whenever he gets near the production unit. The symptoms may appear even though there is no apparent contamination of the surrounding atmosphere." The memorandum notes co-exposure to other chemicals such as vanadium pentoxide, but attributes symptoms to inhalation of phthalic anhydride.

The report also includes a correspondence from 1949 that describes respiratory symptoms observed in workers, including sneezing, nasal congestion, persistent non-productive cough, blood-tinged sputum in severe cases, upper mid-sternal discomfort, and para sternal wheezing or course breath sounds. In contrast to the memorandum from 1962, the 1949 report states, "true allergic bronchial asthmatic findings have not been detected." The report indicates that symptoms are primarily attributed to workers handling dry or powdered phthalic anhydride. Details on the number of workers, duration of exposure, or prevalence of symptoms are not provided.

Overall, the epidemiological evidence of multiple targeted studies in phthalic anhydride-handling resin plants show that phthalic anhydride is a respiratory sensitizer that can cause work-related rhinitis and occupational asthma—particularly in workers experiencing brief, high peak dust exposures during manual reactor loading (Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986). Immunologic and specific inhalation challenge (SIC) data (*i.e.,* results from controlled bronchial provocation tests) support an allergic mechanism in some cases (Wernfors et al., 1986). Four broad screening studies were not designed to include phthalic anhydride-specific testing, and therefore did not identify phthalic anhydride-specific disease (TOMA, 1982, 1981, 1979). Barker et al. (1998) supports an association between respiratory sensitization outcomes and acid anhydrides in general but is not specific to phthalic anhydride. Across studies with outcomes that can be specifically linked to phthalic anhydride exposure,

adverse respiratory outcomes such as asthma are correlated with peak exposures rather than low full-shift averages, indicative of a dose-gradient. The available studies, however, are of limited use for quantitative dose-response analysis due to small samples, cross-sectional designs, confounding, mixed co-exposures, lack robust exposure quantification, limited exposure metrics, and survivor/selection biases.

Nielsen et al. (1988) was preferable to Nielsen et al. (1991) for dose-response because Nielsen et al. (1991), which focused on serum IgE and lung function—relied on endpoints that are less specific to phthalic anhydride sensitization, did not pair immunologic testing with concurrent characterization of short-term peak exposures, and did not include phthalic anhydride–specific SIC; as a result, it was less informative for defining a POD. The TOMA (TOMA, 1982, 1981, 1979) screening studies lacked phthalic anhydride-specific endpoints and exposure data while Barker et al. (1998) shows exposure-response for TMA but not phthalic anhydride. Wernfors et al. (1986) provides strong SIC evidence (including elicitation at 0.5 mg/m^3 for 10 min), but the study reports that exposure monitoring was not concurrent with health testing and was across multiple plants. In contrast, the study by Nielsen et al. (1988) pairs concurrent exposure characterization with immunology and at least one phthalic anhydride SIC in the same worker population. Compared to the other studies mentioned, the study by Nielsen et al. (1988) provides a directly relevant exposure metric (5−30 minute peaks during loading) and clinically significant effects such as asthma correlated with serum specific phthalic anhydride-HSA-specific IgG antibody levels linked to those peaks.

Although there were limitations and uncertainties associated with the study by Nielsen et al. (1988), the study is the most suitable of the available epidemiological studies for consideration as a candidate POD because it reported elevated phthalic anhydride-specific IgG alongside work-related asthma and rhinitis in heavily exposed workers, and contained information to establish a biological gradient. The study was also used by other agencies to set a POD, namely Health Canada (2019) and California OEHHA (2008). Consideration of the study as a candidate POD is discussed further in Section 4.3.1.3.

2671 **Table 4-17. Summary of Respiratory Sensitization Effects Observed in Epidemiological Studies**

Reference	Brief Study Description	NOAEC / LOAEC (mg/m^3)	Effect at LOAEC	Limitations/Uncertainties	Study Quality Rating [a]
(Wernfors et al., 1986)	• Mixed study (cross-sectional study of current workers and retrospective field survey of former workers) in 4 alkyd/unsaturated polyester resin plants. • Combined survey of currently and formerly exposed workers, with clinical testing in a subset. • 118 workers (48 current, 70 former) with ≥2 months of phthalic anhydride exposure • Task-based phthalic anhydride measurements during loading/empty-bag handling ≈3–13 mg/m^3; other tasks <0.3 mg/m^3. • Clinical testing in subsets; bronchial challenge in 2 asthmatics.	• LOAEC Induction (operational): ≈3–13 mg/m^3 during short-term tasks. Elicitation: as low as 0.5 mg/m^3 for 10 min in 1 sensitized worker (another required 6 mg/m^3 for 5 min). • NOAEC not identified for induction of rhinitis/asthma	• ↑ prevalence of work-related rhinitis and asthma (up to ≈25% in loaders) • ↑ nonspecific bronchial hyperreactivity among heavily exposed non-asthmatics (16%)	• Cross-sectional study design (cannot establish incidence or temporality) • Discrepancy in asthma prevalence in the abstract (28%) vs. results (21/118 = 18%) • Insufficient graded exposure for modeling concentration-response • Survivor and reporting biases • Co-exposure to MA and TMA • Small numbers for confirmatory tests: bronchial challenges (n = 2), phthalic anhydride skin tests (n = 11 asthmatics), P-K tests (n = 2)	Low
(Nielsen et al., 1988)	• Cross-sectional study of workers with concurrent exposure monitoring and immunologic testing that evaluated symptoms in 60 workers exposed to phthalic anhydride at two plants in Sweden that manufactured alkyd and unsaturated polyester resins. • 60 workers (35 heavy loaders (n = 28 reactor loaders, n = 7 repair men), 25 low exposure) and 22 male controls. • Peak air concentration of phthalic anhydride during reactor loading exposures ≈6.6 mg/m^3 (range 1.5–17.4) for minutes; 8hr TWA ≈0.4 mg/m^3. • One of five workers with asthma had a positive skin prick test against phthalic anhydride conjugated with HSA	• LOAEC 6.6 mg/m^3 during short loading tasks (peaks up to 17.4 mg/m^3) while the 8-hour TWA was 0.4 mg/m^3. • NOAEC not identified for rhinitis/asthma or immunologic endpoints; effects appeared driven by short-term peaks rather than 8-hour TWAs.	• High prevalence of conjunctivitis and/or rhinitis (69% among heavily exposed) and PA-associated asthma (14% among heavy). • ↑ Phthalic Anhydride-specific IgG in heavily exposed vs low/control • IgG4 detected in 3 asthmatics and 1 with rhinitis.	• Cross-sectional study design (cannot establish incidence or temporality) • No distinguishing between sensitized and newly sensitized workers • Limited number of short-duration personal samples • Small sample size, only assessed current workers • Exposure was characterized by job task (heavy vs. low) with limited short-term measurements • Inconsistent respirator use for phthalic anhydride • Co-exposures to MA, IPA, TMA • Limited linkage of exposure to individuals; intermittent peaks with irregular respirator use • NOAEC could not be defined.	Low-Medium [a]

Reference	Brief Study Description	NOAEC / LOAEC (mg/m^3)	Effect at LOAEC	Limitations/Uncertainties	Study Quality Rating [a]
(Nielsen et al., 1991)	• Cross-sectional study in 2 Swedish plants manufacturing alkyd and/or unsaturated polyester resins. • comparison of currently employed workers with phthalic anhydride exposure versus unexposed controls • 23 male exposed workers (19 regular loaders, 4 with considerable exposure) and 18 unexposed municipal workers matched on age and smoking. • Short-term loading exposures up to 17 mg/m^3 • Evaluated work-related symptoms, total/specific IgE and IgG, spirometry, small-airways indices, TLCO, and 99mTc-DTPA clearance	• LOAEC (short-term): ≈6–7 mg/m^3 (minutes per event) with peaks up to ≈17 mg/m^3 during loading • NOAEC not identified for irritation/asthma	• ↑ prevalence of work-related ocular and nasal irritation (48% conjunctivitis; 39% rhinitis; 22% rhino-conjunctivitis). • Higher total serum IgE and Phthalic anhydride-specific IgG in exposed.	• Cross-sectional study design (cannot establish incidence or temporality) • Small sample size—limited power for asthma and subgroup analyses • Limited task-based sampling and no individual-level long-term or peak exposure metrics • Irregular respirator use that was not accounted for in the dose • Controls had unexpectedly high nonspecific hyperreactivity (potential selection bias); exposure peaks not individually assigned • Study lacked a minimally exposed internal group with quantified peak exposures. • Co-exposures to MA, TMA, IPA, dust, solvents, occasional TDI • NOAEC for irritation/asthma not defined.	Low
(Barker et al., 1998)	• Historical cohort with follow-up health assessment (1992) across 4 UK industrial sites using acid anhydrides • Factories 1, 3, 4: Alkyd/polyester resin manufacture using primarily phthalic anhydride, with MA and trimellitic anhydride TMA also used. • Factory 2: Cushioned flooring manufacture using TMA in printing ink • Quantify exposure–response relations for: sensitization (immediate skin prick test reaction to acid anhydride–human serum albumin [AA–HSA] conjugates) • Current (1992) personal full-shift and task sampling • Retrospective job–time–exposure matrix constructed by hygienists blinded to health outcomes.	TMA LOAEC ≈10 $\mu g/m^3$ (full-shift) TMA NOAEC <10 $\mu g/m^3$ (full-shift)	Associated with significantly ↑ odds of respiratory symptoms	• Study does not provide data-specific to phthalic anhydride, but rather "acid anhydrides" broadly	Medium

Reference	Brief Study Description	NOAEC / LOAEC (mg/m^3)	Effect at LOAEC	Limitations/Uncertainties	Study Quality Rating [a]
	• Manual loading of phthalic anhydride; TMA handled with full respiratory protection				
(TOMA, 1981) (TOMA, 1982) (TOMA, 1979) (TOMA, 1981)	• Series of low quality cross-sectional occupational health studies by Tabershaw Occupational Medicine Associates, P.A. (TOMA) plant that produced phthalic anhydride, polyester resin and some alkyd resins for chemical processors, paint and coatings • The lung, liver, kidney, blood, and skin were the targets of a general screening profile because of the high number of chemicals produced at the plant with non-specific health consequences • There were no reported cases of occupational asthma • Other outcomes that may be related to respiratory sensitization were not evaluated	None identified	None identified	• Cross-sectional study • No clear association between exposure to phthalic anhydride and ↑ rates of allergic sensitization or occupational asthma • Principal occupational findings centered on dermatologic effects (folliculitis/chloracne) consistent with coal tar exposures • Did not report any specific phthalic anhydride exposure measurements or quantitative job-exposure matrix and no resolution of peak exposures that are relevant for sensitization • No phthalic anhydride specific health outcomes • Co-exposure with other anhydrides	Low

TMA = trimellitic anhydride ; MA = maleic anhydride; TOMA = Tabershaw Occupational Medicine Associates; LOAEC = lowest-observed-adverse-effectconcentration; NOAEC = no-observed-adverse-effect concentration; TWA = Time weighted average; HSA = human serum albumin

[a] The study quality rating for Nielsen et al. (1988) of "Low-Medium" reflects the rating of "Low" for "irritation of the upper airways, rhinitis, asthma, phthalic anhydride-induced asthma, chronic productive bronchitis, spirometry" and "Medium" for serum specific IgG antibodies.

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Laboratory Animal Evidence

In animals, there are currently no validated approaches or test methods to evaluate respiratory sensitization. Nevertheless, EPA considered functional outcomes in reasonably available laboratory animal studies to be relevant for the adverse outcome, including evidence of respiratory reactions, changes in lung volume, and changes in airway resistance.

EPA identified six publications evaluating inhalation exposures of phthalic anhydride with experimental animal models (3 of guinea pigs, 2 of rats, 1 of mice) (Arts et al., 2008; IIT Research Institute, 1996; Blaikie et al., 1995; IIT Research Institute, 1995; Sarlo et al., 1994; Sarlo and Clark, 1992). Although there are currently no widely accepted animal models for respiratory sensitization, in some cases indications of respiratory sensitization in animal inhalation studies can be considered to support a weight of evidence hazard identification. Available studies report effects consistent with respiratory irritation and/or sensitization across species (*i.e.,* rats, mice, guinea pigs) and that are coherent with findings from human occupational studies described above. The majority of identified studies employed sensitization and challenge paradigms, and reported effects on pulmonary function, immune system response (*e.g.,* increased serum antibody levels), and respiratory tract histopathology (*e.g.,* hemorrhagic foci). Several of the available studies also provide evidence of sensitization across routes of exposure (*e.g.,* dermal route of entry and respiratory sensitization upon challenge). Available studies are summarized in Table 4-18 and briefly discussed below; more detailed study summaries can be found in Appendix A.3. The evidence is discussed in detail in the current section as evidence for the adverse outcome in AOP 39.

The IIT Research Institute (1996) exposed 10 male and 10 female SD rats to aerosolized particles of phthalic anhydride via whole body inhalation at a target concentration of 0.5 mg/m^3 for 6 hours/day for 5 days (TWA = 0.525 mg/m^3; range: 0.404–0.746 mg/m^3 across 5 days). The authors do not provide quantitative measures of size (*i.e.,* MMAD) or distribution of the particles. The rats were rested for three weeks and then challenged with 0.481 mg/m^3 of phthalic anhydride for 6 hours. A control group of rats was also included that was not initially exposed to phthalic anhydride or challenged.

Increased incidences of hemorrhagic lung foci were observed in rats exposed to and then challenged with phthalic anhydride (*i.e.,* 7/10 males; 3/10 females had ≥10 foci/lung compared to 0/20 animals in the control group). Serum levels of phthalic-anhydride-specific IgG antibodies were also observed in rats exposed and challenged with phthalic anhydride. Lungs from three animals from each group were examined microscopically. In rats exposed to and challenged with phthalic anhydride, observations included alveolar hemorrhage (2/3 rats), parabronchial lymphoid hyperplasia (1/3 rats), and perivascular acute and chronic inflammation (1/3 rats). No microscopic lung lesions were observed in the control group. Despite several limitations (detailed in Appendix A.3 and Table 4-18), the study provides additional evidence that phthalic anhydride is a respiratory sensitizer. An abbreviated version of these studies is also found in a report by Amoco Chemical Company (1988). The study was rated *Uninformative*.

In a study of similar design, Sarlo et al. (1992) exposed female Harley guinea pigs to air concentrations of 0, 0.05 to 0.2, or 0.6 to 6 mg/m^3 phthalic anhydride dust for 3 hours per day for 5 consecutive days, rested animals for two weeks, and then challenged animals with a single 30-minute inhalation exposure to aerosolized phthalic anhydride-guinea pig serum albumin (GPSA) conjugate. Dust concentrations were reported as a range due to the day-to-day difficulty in controlling the dust levels in the exposure chambers. All guinea pigs in the high exposure group had significant respiratory reactions (defined as >46% increases in respiratory rate and/or >50% increase in breath peak height) and no statistically significant changes in serum IgG and antibody titers specific (IgG1a) for phthalic anhydride-GPSA conjugate. The study was rated *Medium*.

In a subsequent study by the same authors (Sarlo et al., 1994), female Hartley smooth-haired guinea pigs were exposed via whole-body inhalation to 0.5, 1, or 5 mg/m^3 phthalic anhydride dust (MMAD ≤ 4 µm) for 3 hours per day for 5 consecutive days. Animals were then rested for 2 weeks and challenged with a single 30-minute inhalation exposure to either phthalic anhydride dust (5 mg/m^3), or with phthalic anhydride-GPSA conjugate (2 mg/m^3). A dose-dependent increase in IgG antibody levels was observed in sera collected 2 days before challenge in animals exposed to phthalic anhydride dust. In animals sensitized with 0.5 mg/m^3 phthalic anhydride dust and challenged with 5 mg/m^3 phthalic anhydride-GPSA conjugate, an increase in plethysmograph pressure and breathing rate was observed in parallel with increases in serum levels of IgG and IgG1a, but not IgE. In animals initially exposed to 5 mg/m^3 phthalic anhydride dust and challenged with 5 mg/m^3 phthalic anhydride dust, an increase in the incidence of hemorrhagic lung foci was observed in all animals, in parallel with increases in serum levels of IgG1a (but not IgE) antibodies specific to phthalic anhydride. The study was rated *Medium*.

Another study by the IIT Research Institute (1995) exposed four male SD rats to 486 mg/m^3 phthalic anhydride dust (MMAD = 4.95 ± 1.87 µm) via head-only inhalation for at least 10 minutes and then evaluated respiratory rate to determine if short-term exposure produces pulmonary sensory irritation. No changes in average respiratory rates were observed during or immediately after exposure, indicating phthalic anhydride dust did not produce pulmonary sensory irritation. The study was rated *High*.

In a series of publications by Arts et al. (2008) and De Jong et al. (2009), male BALB/c mice were exposed to 0 or 15 mg/m^3 phthalic anhydride (MMAD = 2.6 ± 4.4 µm) via nose-only inhalation for 6 hours per day for 3 consecutive days. Three days after the last exposure, significant increases in *ex vivo* lymphocyte proliferation were observed in the mandibular lymph nodes (SI = 2.9 ± 0.52), but not the auricular lymph nodes. In parallel, enlargement of the mandibular lymph nodes was observed. Histopathologic lesions were observed in the nasal cavity and larynx, including inflammatory lesions and squamous metaplasia/hyperplasia of the nasal cavity and larynx; however, the results for control animals were not provided. Changes in cytokines in mandibular lymph node cells stimulated with Conclavin A were also observed, including increases in IL-4, IL-10, and IFN-γ, but not IL-12. The study (Arts et al., 2008) was rated *High*.

Blaikie et al. (1995) provide data from four studies conducted across two laboratories demonstrating that single intradermal injections of 0.03 to 0.3% phthalic anhydride in female Dunkin-Hartly guinea pigs are sufficient to result in an allergic response following 15-minute inhalation challenges with phthalic anhydride-GSA conjugate dust 22 days after the initial injection. In three of four studies, an increased number of animals per group with a severe pulmonary response (*i.e.,* ≥70% decrease in respiratory rate during 15-minute challenge period) was observed in groups that received a dermal induction dose of phthalic anhydride and inhalation challenge compared to groups that did not. In the one study where no pulmonary response was observed, study authors report rapid oxidation of phthalic anhydride in air, while subsequent studies where a positive pulmonary response was observed were conducted with dry air or argon gas to overcome stability issues. Prior to inhalation challenge, animals that had been administered the single dermal induction dose had elevated serum levels of anti-phthalic anhydride-GPSA IgG1 antibody on study day 19 in all four studies. The study was rated *Uninformative*.

Table 4-18. Summary of Respiratory Effects Observed in Animal Toxicology Studies Following Inhalation Exposure to Phthalic Anhydride

Brief Study Description	NOAEC/ LOAEC (mg/m^3)	Effect at LOAEC	Remarks	Study Quality Rating[a]
Female Hartley guinea pigs (5–6/group) were exposed (whole-body) to 0.05–0.2 mg/m^3 or 0.6–6 mg/m^3 phthalic anhydride dust for 3 h/day, 5 consecutive days (MMAD = 5.8–9.8 µm). Controls (8 total) exposed to filtered air. Two weeks after the final exposure, animals challenged with aerosolized phthalic anhydride-GPSA conjugate for 30 min (Sarlo and Clark, 1992)	0.05–0.2 / 0.6–6 (phthalic anhydride-GPSA conjugate challenge)	↑ Respiratory reactions; ↑ IgG and allergic antibodies (IgG1a) to phthalic anhydride-GPSA	Considerations: - Evaluated outcomes limited to respiratory rate and serum antibody levels - Exposure characterization concerns resulted in difficulty maintaining constant exposure - No group challenged with phthalic anhydride dust alone (*i.e.,* just phthalic anhydride-GPSA conjugate) - Respiratory reaction defined as an increase in respiratory rate >46% and/or increase in breath peak height >50% - GSD not reported or calculatable	Medium
Male and female SD rats (n = 10/group) exposed via whole-body inhalation to 0.5 mg/m^3 phthalic anhydride (target concentration) for 6 h/day for 5 consecutive days (measured concentrations: TWA = 0.525 mg/m^3; range: 0.404–0.746 mg/m^3 across 5 day), rested for 3 weeks, then challenged with 0.5 mg/m^3 phthalic anhydride for 6 h (measured concentration: 0.481 mg/m^3. A nonexposed control was also included (IIT Research Institute, 1996)	ND/ 0.5	↑ phthalic anhydride-specific IgG antibodies in serum; ↑ incidence of hemorrhagic lung foci (7/10 males 3/10 females had ≥10 foci/lung) and challenged with 0.5 mg/m^3 phthalic anhydride	Considerations: - Insufficient detail on exposure characterization provided (*e.g.,* particle size or distribution) - Small sample sizes (n = 1–3/sex/group) for microscopic evaluations of the lung, which included - Author-reported evidence of differences between control and exposure groups (*i.e.,* controls were not fasted)	Uninformative
Female Hartley guinea pigs (8–16/group) exposed via whole-body inhalation to 0 (filtered air), 0.5, 1, or 5 mg/m^3 phthalic anhydride dust for 3 h/day, 5 consecutive days (measured concentrations: 0.55, 1.27, 5.57 mg/m^3; MMAD (± GSD) = 3.12 µm ± 2.02 , 3.26 µm ± 1.96, 3.91 µm ± 2.08). Two weeks after the last exposure, control and high-dose guinea pigs challenged with 5 mg/m^3 phthalic anhydride dust for 30 minutes, while animals in all treatment groups were challenged with 2 mg/m^3 aerosolized phthalic anhydride-GPSA conjugate for 30 minutes dust (Sarlo et al., 1994)	ND / 0.5 (phthalic anhydride-GPSA conjugate challenge)	↑ plethysmograph pressure, ↑ breathing rate, ↑ serum IgG & IgG1a	Unaffected outcomes: - Serum IgE Considerations: - ↑ plethysmograph pressure in 1/8, 1/8, 3/8 animals challenged with phthalic anhydride-GPSA across dose groups - ↑ breathing rate 1/8, 0/8, 4/8 animals challenged with phthalic anhydride-GPSA across dose groups - ↑ serum IgG and IgG1a antibody (≥0.5 mg/m^3) - ↑ incidence of hemorrhagic lung foci in 8/8 exposed and challenged with 5 mg/m^3 phthalic anhydride dust (histopathology evaluated in control and high-dose groups)	Medium

Brief Study Description	NOAEC/ LOAEC (mg/m^3)	Effect at LOAEC	Remarks	Study Quality Rating[a]
	ND / 5 (phthalic anhydride dust challenge)	↑ serum IgG; ↑ incidence of hemorrhagic lung foci in 8/8 animals exposed and challenged with 5 mg/m^3 phthalic anhydride dust	Unaffected outcomes: - Phthalic anhydride dust challenge: Respiratory rate & plethysmograph pressure Considerations: - Dose-dependent ↑ in serum IgG -Histopathology evaluated in control and high-dose groups	Medium
Male BALB/c mice (6/group) exposed (head/nose only) for 3 consecutive days for 45, 90, 180, or 360 min/day to 0 (acetone) or 15 mg/m^3 (target concentration) phthalic anhydride (measured concentration = 14.2 mg/m^3; MMAD (±GSD) = 2.6 µm ± 4.4). Following 2 rest days, cell proliferation was evaluated in the draining mandibular lymph nodes & the respiratory tract was examined microscopically in the 360 min/day group; cytokine production was also measured (De Jong et al., 2009; Arts et al., 2008)	ND / 15	Histopathology of nasal tissues & larynx; ↑ proliferation in mandibular LNs	- ↑ proliferation of lymphocytes in mandibular lymph nodes (all exposure durations) - ↑ incidence of histologic findings in nasal cavity (slight to moderate mixed inflammatory cell infiltration & slight squamous metaplasia/hyperplasia) and larynx (slight squamous metaplasia/hyperplasia) Unaffected outcomes: - Proliferation in auricular lymph nodes Considerations - Histopathology not reported for control animals - Clinical signs (piloerection, hunched posture, blepharospasm, dyspnea, sluggishness) - Slight to moderate ↓ BW (90, 180, 360 min groups)	High
Male SD rats (n = 4) exposed to phthalic anhydride dust (486 mg/m^3; MMAD = 4.95 µm; GSD = 1.87) via head-only inhalation for at least 10 mins (0.165 h) on a single day (IIT Research Institute, 1995)	486 / ND	NA	Observed Effects: - None Unaffected Outcomes: - Respiratory rate (measured via plethysmograph over a 3-minute period) Considerations: - Limited outcomes evaluated (objective of study to determine concentration of test substance that would decrease respiratory rate over concern for potential sensory irritation response in the rat)	High

Brief Study Description	NOAEC/ LOAEC (mg/m^3)	Effect at LOAEC	Remarks	Study Quality Rating[a]
Laboratory 1; Experiment 1: Dunkin-Hartley guinea pigs (8/group) with a single 100 µL intradermal injection of 0, 0.03, 0.1, or 0.3% phthalic anhydride in 6% acetone in corn oil vehicle. Serological analysis was performed on Day 19 and animals were challenged via nose-only inhalation with phthalic anhydride in argon gas on day 22 (0 or 11–29 mg/m^3; MMAD: 3.79–4.81 µm) for 15 minutes (Blaikie et al., 1995) [b]	ND / 11–29	↑ severe pulmonary response; ↑ # of animals with positive PCA result; ↑ serum IgG1serological response (PCA assay)	- Severe pulmonary response in 4/8 animals injected with 0.3% phthalic anhydride - Positive PCA result in 0/8, 2/8, 6/8, 7/8 animals across injection groups Considerations: - Exposure concentration reported as a range	Uninformative
Laboratory 1; Experiment 2: Dunkin-Hartley guinea pigs (7–8/group) with a single 100 µL intradermal injection of 0, 0.03, 0.1, or 0.3% phthalic anhydride in 6% acetone in corn oil vehicle. Serological analysis was performed on Day 19 and animals were challenged via nose-only inhalation with phthalic anhydride in dry air on day 22 (0 or 9-48 mg/m^3; (MMAD: 0.61-18.02 µm) for 15 minutes (Blaikie et al., 1995)[b]	ND / 9–48	↑ severe pulmonary response; ↑ # of animals with positive PCA result; ↑ serum IgG1	- Severe pulmonary response in 0/8, 1/7, 1/8, and 3/8 animals across injection groups - Positive PCA result in 0/8, 7/7, 8/8, 8/8 animals across injection groups Considerations: - Moderate response = ↑ in respiration rate to 130% or more of normal background rate within challenge period - Severe response = ↓ in respiratory rate to 70% or less of normal background rate within challenge period - Large variance in exposure (*i.e.,* avg concentration and MMAD) for phthalic anhydride in dry air exposure	Uninformative
Laboratory 2; Experiment 1: Dunkin-Hartley guinea pigs (8–12/group) with a single 100 µL intradermal injection of 0 or 0.3% phthalic anhydride in 6% acetone in corn oil vehicle. Serological analysis was performed on Day 19 and animals were challenged via nose-only inhalation with 0 or 44 mg/m^3 of phthalic anhydride (MMAD ±SD: 5.92 ±1.64 µm) in air on day 22 (Blaikie et al., 1995) [b]	ND / 44	↑ # of animals with positive PCA result; ↑ serum IgG1	- Positive PCA result in 1/8, 12/12 animals across injection groups Unaffected Outcomes - Pulmonary response	Uninformative

Brief Study Description	NOAEC/ LOAEC (mg/m^3)	Effect at LOAEC	Remarks	Study Quality Rating[a]
Laboratory 2; Experiment 2: Dunkin-Hartley guinea pigs (7–12/group) with a single 100 µL intradermal injection of 0 or 0.3% phthalic anhydride in 6% acetone in corn oil vehicle. Serological analysis was performed on Day 19 and animals were challenged via nose-only inhalation with 0, 52 mg/m^3 of phthalic anhydride (MMAD ± SD: 4.71 ± 1.62 µm) in air on day 22 (Blaikie et al., 1995) [b]	ND / 52	↑ severe pulmonary response; ↑ serum IgG1	- Severe pulmonary response in 1/8 and 7/12 animals across injection groups Considerations: - Moderate response = ↑ in respiration rate to 130% or more of normal background rate within challenge period - Severe response = ↓ in respiratory rate to 70% or less of normal background rate within challenge period - PCA analysis not conducted	Uninformative

↓ = statistically significant decrease; ↑ = statistically significant increase; GPSA: guinea pig serum albumin; GSD = geometric standard deviation; Ig = immunoglobulin; NA = not applicable; ND = no data; LOAEC = lowest-observed-adverse-effect-concentration; MMAD = mass median aerodynamic diameter; NOAEC = no-observed-adverse-effect concentration; PCA = passive cutaneous anaphylaxis

[a] SQE conducted according to TSCA systematic review process as described in the *Draft Systematic Review Protocol for Phthalic Anhydride* (U.S. EPA, 2026j).

[b] (Blaikie et al., 1995) reported results of two experiments conducted in two separate laboratories; in all four experiments, the pulmonary response (*i.e.,* no. of animals with "no response" "moderate response" or "severe response") was determined after challenge with phthalic anhydride-GSA conjugate at the indicated concentrations and MMAD 21 days after intradermal injection with phthalic anhydride; A severe response was defined as a decrease in respiratory rate to 70% or less of normal background rate within challenge period; moderate response was defined as an increase in respiration rate to 130% or more of normal background rate within challenge period; no response was defined as changes in respiration rate within 71–129% of the normal background rate within the challenge period.

[c] (Sarlo and Clark, 1992) defined a significant respiratory reaction as "diaphragmatic contractions occurring at a minimum of every 36 to 40 normal breaths over an observation period of 10 minutes."

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4.3.1.2 Evidence Integration Conclusions and Weight of Scientific Evidence: Respiratory Sensitization

EPA applied AOP 39 as an organizing framework to integrate evidence from multiple streams of evidence including mechanistic data from *in chemico* and *in vitro* assays for the potential for exposure to phthalic anhydride to result in respiratory sensitization. Evidence from multiple KEs within AOP 39 support the conclusion that phthalic anhydride is a respiratory sensitizer. Phthalic anhydride tested positive for KE 1 in three of three assays (2 DPRA and 1 kDPRA); two of three assays for KE 3 including GARDair (NTP, 2026) and one of the two ALIsens assays (Invitrolize, 2024; Chary et al., 2019); and KE 4 (respiratory LLNA by Arts et al. (2008)).

With regards to the adverse outcome, EPA identified consistent evidence of respiratory sensitization from human occupational data, as well as *in chemico, in vitro,* and *in vivo* data, as summarized in Section 4.3.1.1.5. The combined human and animal evidence supports that phthalic anhydride elicits an allergic respiratory hypersensitivity response and is a respiratory sensitizer. Across human studies, two of Medium quality (Barker et al., 1998; Nielsen et al., 1988) and two low quality studies (Nielsen et al., 1991; Wernfors et al., 1986) that were used qualitatively to add to the weight of scientific evidence, provide consistent evidence of work-related asthma (including immediate, late, or dual-type reactions).. Increases in serum levels of specific IgG antibodies against haptenized phthalic anhydride-HSA accompanied work-related respiratory disease in two studies (Nielsen et al., 1991; Nielsen et al., 1988), positive skin prick tests were reported in three studies (Barker et al., 1998; Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986), and positive bronchial provocation tests (specific inhalation challenges) to short-term phthalic anhydride exposure (5–10 minutes) were obtained in two volunteers with positive skin prick tests (Wernfors et al., 1986). Evidence of a biological gradient was reported by Nielsen et al. (Nielsen et al., 1991) and Barker et al. (Barker et al., 1998), where higher levels of exposure to phthalic anhydride (or more general acid anhydrides in Barker et al.) correlated with increased prevalence of symptoms and elevated specific IgG levels.

Consistent with the human evidence, six experimental studies of laboratory animals (3 of guinea pigs, 2 of mice, 1 of rats) provide consistent evidence of respiratory sensitization (Section 4.3.1.1.5). Two studies received High study quality rating (Arts et al., 2008; IIT Research Institute, 1995); Two studies received a Medium rating (Sarlo et al., 1994; Sarlo and Clark, 1992); and two received an Uninformative rating (IIT Research Institute, 1996; Blaikie et al., 1995). Study quality ratings for these studies are summarized in Table 4-18 and Table 4-19. Notably, effects were observed consistently across species and studies conducted at multiple laboratories by multiple research groups. Changes in lung function and/or increases in breathing rate were observed following inhalation exposure to phthalic anhydride in two studies of guinea pigs (Sarlo et al., 1994; Sarlo and Clark, 1992). Increased lymphocyte proliferation in the mandibular lymph node was reported in one study of mice exposed to phthalic anhydride (De Jong et al., 2009; Arts et al., 2008), while increased incidence of lung histopathology (*e.g.,* hemorrhagic foci) was observed in one study of each rats, mice, and guinea pigs (De Jong et al., 2009; Arts et al., 2008; IIT Research Institute, 1996; Sarlo et al., 1994). Finally, increased serum levels of IgG and IgG1a, including antibodies specific for phthalic anhydride-GPSA were reported in two studies of guinea pigs (Sarlo et al., 1994; Sarlo and Clark, 1992) and one study of rats (IIT Research Institute, 1996).

Consistent increases in serum IgG across human and animal studies were noted, and both Nielsen et al. (1988) and Sarlo et al. (1994) studies identified LOAECs based on increased phthalic anhydride-specific IgG levels. The totality of reasonably available human, animal, and *in vitro* evidence for phthalic anhydride is coherent and consistent with respiratory sensitization (including post-exposure occupational

asthma cases) and aligns with AOP 39, adding to the strength of the evidence. Additionally, coherence across available epidemiologic, i*n vivo* experimental animal, and *in vitro* data that indicate phthalic anhydride is a respiratory sensitizer increase EPA's confidence in the conclusion that phthalic anhydride is a respiratory sensitizer.

EPA also considered human and animal data for the analog TMA as part of its hazard characterization to supplement the available data for phthalic anhydride (Appendix B). Consistent with human epidemiologic studies of phthalic anhydride, epidemiologic studies of workers exposed to TMA have also reported increased prevalence of occupational asthma and increases in serum antibodies against TMA-HSA (Appendix B). Further, across six experimental inhalation studies of TMA with SD rats observed effects were consistent with those observed in studies of phthalic anhydride, including dose-related increases in lung histopathology (*e.g.,* hemorrhagic foci), and serum antibody levels against TMA-RSA (Appendix B). Given the consistency with phthalic anhydride, findings from human epidemiologic and experimental animal studies of TMA increase EPA's confidence in the conclusion that phthalic anhydride is a respiratory sensitizer.

In addition, both inhalation and dermal exposure can contribute to the induction of respiratory sensitization, as described above. This has been shown in animal models following dermal induction and inhalation challenge with phthalic anhydride (Fukuyama et al., 2010; Blaikie et al., 1995; Sarlo and Clark, 1992), but has not been evaluated in humans. For dermal exposures, EPA preliminarily concluded in its weight of scientific evidence assessment (Section 4.2.1.4) that phthalic anhydride is a dermal sensitizer. EPA also considered dermal sensitization as part of its weight of scientific evidence conclusions for respiratory sensitization potential of phthalic anhydride following inhalation exposure, consistent with the approach taken by existing assessments.

Phthalic anhydride is also classified as an irritant. However, irritation occurs at higher exposure levels than sensitization and results from direct tissue damage response with an immediate effect. In contrast, sensitization is a delayed, specific immune-mediated process that can be triggered by lower exposure levels. Multiple lines of evidence support the induction of an immunological response (*i.e.,* sensitization) following exposure to phthalic anhydride, supporting that sensitization is the more sensitive endpoint. Consistent with this, the work-related asthma reported by Nielsen et al. (Nielsen et al., 1991; 1988) is more likely immune-mediated given detection of antibodies specific to haptenized phthalic anhydride in affected workers. Similarly, from the animal evidence, three studies reported increases in IgG antibodies specific to haptenized phthalic anhydride, including two studies of guinea pigs (Sarlo et al., 1994; Sarlo and Clark, 1992) and one study of rats (IIT Research Institute, 1996). Increases in serum levels of specific antibody were also reported in human and animal studies of the analog, TMA (Appendix B), lending further support for an immune-based mechanism.

Overall, EPA concludes that there is robust evidence that phthalic anhydride is a respiratory sensitizer. EPA considers respiratory sensitization for dose-response assessment in Section 4.3.1.3. Notably, EPA's conclusion is consistent with that of other regulatory and authoritative bodies, including OECD (2005), Health Canada (2019), Australia NICNAS (2013), and ACGIH (2025).

Table 4-19. Summary of Strengths and Uncertainties of Evidence Supporting Respiratory Effects of Phthalic Anhydride

Available Studies	Strengths of Evidence	Uncertainties of Evidence
Human Evidence – phthalic anhydride (see Section 4.3.1.1.5 for further details)		
• 5 occupational exposure studies (2 Medium-, 3 Low-quality) evaluated respiratory effects following inhalation exposure to phthalic anhydride (Barker et al., 1998; Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986; TOMA, 1982, 1981, 1979) [a]	• Consistent evidence of work-related asthma or respiratory symptoms in 4 studies, including immediate, late, or dual type asthmatic reactions (Barker et al., 1998; Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986) • Increased serum levels of specific IgG against phthalic anhydride-HSA in 2 studies (Nielsen et al., 1991; Nielsen et al., 1988) (outcome not evaluated in 3 remaining studies) • Positive skin prick tests associated with occupational asthma in 3 studies (Barker et al., 1998; Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986) (outcome not evaluated in 2 remaining studies) • Positive bronchial provocations obtained by short-term (5–10 minute) exposure to phthalic anhydride in 2 volunteers with positive skin prick tests in 1 study (Wernfors et al., 1986) (outcome not evaluated in 4 remaining studies) • Medium quality studies (Barker et al., 1998; Nielsen et al., 1988)	• Other known respiratory sensitizers (*e.g.,* maleic anhydride, TMA) also reported to be in use at facilities in all 5 studies • Variable respirator use reported when handling phthalic anhydride and other respiratory sensitizers • Studies generally did not control for confounding factors, such as age, sex, smoking status
In vivo experimental animal evidence – phthalic anhydride (see Section 4.3.1.1.5 for further details)		
• Six studies (3 in guinea pigs [1 High, 2 Medium]; 2 in mice [1 High, 1 Uninformative]; ;1 in rats [Uninformative]) provide data on the respiratory and/or immune effects of phthalic anhydride following inhalation exposure. • Four studies adhere to a sensitization paradigm (*i.e.,* include an induction and elicitation/challenge phase) (IIT Research Institute, 1996; Blaikie et al., 1995; Sarlo et al., 1994; Sarlo and Clark, 1992), while 2 studies provide data on lung histopathology and lymphocyte proliferation (Arts et al., 2008) and measures of lung function (IIT Research Institute, 1996, 1995).	• Changes in lung function and/or increases breathing rate in 2 studies of guinea pigs (Sarlo et al., 1994; Sarlo and Clark, 1992) • Increase in lymphocyte proliferation in mandibular lymph nodes in 1 study of mice (De Jong et al., 2009; Arts et al., 2008), which supports KE 4 of AOP 39, the AOP under development for respiratory sensitization • Increased incidence of lung histopathology (*e.g.,* hemorrhagic foci) in 1 study of each rats, mice, and guinea pigs (Johnson et al., 2022; De Jong et al., 2009; Arts et al., 2008; IIT Research Institute, 1996; Sarlo et al., 1994) • Increased serum levels of IgG and IgG1a, including antibodies specific for phthalic anhydride-GPSA in 2 studies of guinea pigs (Sarlo et al., 1994; Sarlo and	• No validated *in vivo* test methods are available for identifying respiratory sensitizers (as outlined in (OECD, 2025a)) • Lack of studies available to ascertain dose-response • Imprecision in exposure measurement in some studies (*i.e.,* (Sarlo and Clark, 1992))

Available Studies	Strengths of Evidence	Uncertainties of Evidence
	Clark, 1992) and one of rats (IIT Research Institute, 1996) • Evidence of cross-route sensitization (*i.e.,* dermal induction, inhalation challenge) in 2 studies of guinea pigs and 1 study of mice (Fukuyama et al., 2010; Blaikie et al., 1995; Sarlo and Clark, 1992) • Findings consistent across species (*i.e.,* rats, guinea pigs, mice) and studies conducted at multiple laboratories • Coherence with observations from human evidence as well as *in vitro* evidence • Biological plausibility – evidence is consistent with that which would be expected from a sensitizer • High- (Arts et al., 2008; IIT Research Institute, 1995); and Medium-quality studies (Sarlo et al., 1994; Sarlo and Clark, 1992).	
in vitro evidence – phthalic anhydride (see Section 4.3.1.1.5 for further details)		
• Phthalic anhydride has been evaluated in 3 *in vitro* respiratory sensitization assays including the GARDair assay (NTP, 2026) and ALIsens model (Invitrolize, 2024; Chary et al., 2019) • Three DPRA assays available (Wareing et al., 2017; Bauch et al., 2012; Gerberick et al., 2004)	• Phthalic anhydride was classified as a respiratory sensitization in 1 of 2 ALIsens assays and in the GARDair assay • Positive results in all three DRPA assays provide support for KE 1 in AOP 39	
Human Evidence – trimellitic anhydride (analog) (see Section 4.3.1.2 for further details)		
• Twenty four occupational exposure studies (Grammer et al., 2002; Grammer et al., 2000; Grammer et al., 1999; Barker et al., 1998; Grammer et al., 1998; Piirilä et al., 1997; Gerhardsson et al., 1993; Grammer et al., 1993; Gerhardsson et al., 1992; Grammer et al., 1992; Boxer et al., 1987; Letz et al., 1987; Rosenman et al., 1987; Topping et al., 1986; McGrath et al., 1984; Bernstein et al., 1983; Bernstein et al., 1982; Rivera et al., 1981; Sale et al., 1981; Ahmad et al., 1979; Patterson et al., 1979; Fawcett et al., 1977; Rice et al., 1977; Zeiss et al., 1977) have evaluated respiratory effects following inhalation exposure to TMA.	• Consistent evidence of work-related asthma and other respiratory outcomes (*e.g.,* pulmonary diseases anemia syndrome) as well as antibody levels (IgE and/or IgG subclasses) specific to TMA-HSA in 8t studies (Grammer et al., 1998; Gerhardsson et al., 1992; Letz et al., 1987; McGrath et al., 1984; Rivera et al., 1981; Sale et al., 1981; Ahmad et al., 1979; Patterson et al., 1979; Zeiss et al., 1977) • Three studies provide evidence to inform an exposure gradient for TMA exposure and respiratory and/or immunogenic outcomes (*i.e.,* TMA-HSA antibody levels) (Grammer et al., 1999; Barker et al., 1998; Grammer et al., 1992)	• Other known respiratory sensitizers (*e.g.,* maleic anhydride, phthalic anhydride) also reported to be in use at facilities (Barker et al., 1998) • The majority of studies lack longitudinal data • Some studies rely on self-reported symptoms on a questionnaire (*e.g.,* (Barker et al., 1998; Gerhardsson et al., 1992))

Available Studies	Strengths of Evidence	Uncertainties of Evidence
	• Coherence with *in vivo* experimental animal evidence as well as existing understanding of respiratory sensitizers further supports causal inference. • Implementation of workplace control measures results in decreased TMA exposure and improved health outcomes in one study with longitudinal follow up (Boxer et al., 1987)	
In vivo experimental animal evidence – trimellitic anhydride (analog) (see Section 4.3.1.2 for further details)		
• Six studies (all in SD rats) provide data on the respiratory and/or immune effects of TMA following inhalation exposure (Leach et al., 1989; Zeiss et al., 1989; IIT Research Institute, 1988; Zeiss et al., 1988; Leach et al., 1987; Zeiss et al., 1987).	• Consistent increases in relative lung weight and/or volume; incidence of gross and microscopic lung findings (*e.g.,* hemorrhagic lung foci, multifocal lobular bronchopneumonia); and serum antibody levels against TMA-RSA in all studies • Effects on relative lung weight/volume, incidence of gross and microscopic lesions in lung, and serum antibody levels were dose-related in 2-, 6.5-, and 13-week dose-response studies of TMA • Evidence that lung and antibody effects were reversible in studies that included recover groups, but recurred upon challenge with a single exposure to TMA • Coherence with observations from TMA human evidence • Biological plausibility – evidence is consistent with that which would be expected from a sensitizer	• Lack of studies in species other than rat

AOP = adverse outcome pathway; Ig = immunoglobulin; HSA = human serum albumin; RSA = rat serum albumin; TMA = trimellitic anhydride

[a] Study quality evaluation results for human evidence: Two studies received a Medium study quality rating (Barker et al., 1998; Nielsen et al., 1988), all other studies received a Low study quality rating (Nielsen et al., 1991; Wernfors et al., 1986; TOMA, 1982, 1981, 1979)

[b] Study quality evaluation results for animal evidence: Two studies received High study quality rating (Arts et al., 2008; IIT Research Institute, 1995); Two studies received a Medium rating (Sarlo et al., 1994; Sarlo and Clark, 1992); and 2 received an Uninformative rating (IIT Research Institute, 1996; Blaikie et al., 1995).

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4.3.1.3 Dose-Response Assessment: Respiratory Sensitization

EPA identified respiratory sensitization as the key endpoint for inhalation POD derivation. EPA considered studies from the pool of reasonably available data on humans and animals exposed to phthalic anhydride for dose-response analysis, including studies in exposed workers. EPA also considered data from an analog, TMA. Altogether, EPA considered six studies from experimental laboratory animals exposed to phthalic anhydride via inhalation, six studies of exposed workers, and six studies of animals exposed to TMA. Each evidence stream, including its strengths and uncertainties, are summarized in Table 4-19.

EPA considered the most suitable candidate POD from each evidence stream in Section 4.3.1.3.1 (Human Evidence), Section 4.3.1.3.2 (Animal Evidence for Phthalic Anhydride), and Section 4.3.1.3.3 (Animal Evidence for TMA) as options for deriving draft PODs. EPA also evaluated existing inhalation hazard values including occupational exposure limits (OELs) from other organizations and existing assessments of phthalic anhydride (Section 4.3.1.3.4).

4.3.1.3.1 Proposed POD from Human Evidence

As discussed in Section 4.3.1.1.5, EPA identified five epidemiological studies of workers occupationally exposed to phthalic anhydride via inhalation (Barker et al., 1998; Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986; TOMA, 1982, 1981, 1979). Two of the studies received a Medium quality rating (Barker et al., 1998; Nielsen et al., 1988) and three (Nielsen et al., 1991; Wernfors et al., 1986; TOMA, 1982, 1981, 1979) were of Low quality and used qualitatively as part of the weight of scientific evidence.

Only one study (Nielsen et al., 1988) was considered suitable to derive a candidate POD. While this study had some limitations and uncertainties including exposure characterization and exposure confounding, it provided empirical, concurrent exposure data specific to phthalic anhydride, including short-term peak task concentrations and an 8-hour TWA, compared to the other available studies. Nielsen et al. (1988) pairs concurrent exposure characterization with immunologic testing and clinical outcomes in the same worker population. In contrast, Barker et al. (1998) examined general "acid anhydride" exposure, and the TOMA screening studies (TOMA, 1982, 1981, 1979) lacked phthalic anhydride-specific endpoints and exposure data. Wernfors et al. (1986) provides strong SIC evidence (including elicitation at 0.5 mg/m^3 for 10 minutes), but exposure monitoring was not as closely correlated with health testing and monitoring spanned multiple plants. Nielsen et al. (1991) reports higher prevalence of work-related respiratory symptoms and elevated levels of phthalic anhydride-specific IgG antibodies in serum, but is overall less informative for defining a POD, as reflected by its study quality rating of *Low.*

The study by Nielsen et al. (1988) supports a LOAEC of 0.4 mg/m^3 (8-hour TWA) based on a clear contrast between exposure groups: the "heavily" exposed reactor loaders (high exposure) had a higher prevalence of asthma, conjunctivitis, and rhinitis and showed elevated phthalic anhydride–HSA-specific IgG, whereas the "slightly" exposed "other work" group (low exposure)—with 30-minute peaks below the limit of detection (0.1 mg/m^3)—exhibited substantially fewer respiratory/ocular symptoms and little to no phthalic anhydride–HSA-specific IgG reactivity. The authors determined the 8-hour TWA of 0.4 mg/m^3 as equivalent to full-shift exposure for the "heavily" exposed group which consisted of workers categorized as reactor loaders experiencing a mean 30-minute peak of 6.6 mg/m^3 during manual loading, assuming low exposures during non-loading periods (TWA measured concentrations were 6.2 mg/m^3 over 1.9 hours in one plant and 6.8 mg/m^3 over 6 hours in the second plant). In workers assigned to "other work," the 30-minute peak exposure measured over 12 hours was below the limit of detection

(0.1 mg/m^3). The "other work" group, which was also considered the "slightly" exposed group, serves as the comparator; therefore, no NOAEC was determined. As described in Section 4.3.1.1.5, uncertainties include variation in exposure levels during a full shift, cross-sectional design, co-exposures to other anhydrides, irregular respirator use, small case numbers, and lack of historical control data.

EPA recognizes that some factors could bias estimates toward the null (*e.g.,* healthy worker effect) while others could bias them away from the null (*e.g.,* co-exposures to other acid anhydrides such as TMA), making the net direction of bias uncertain. Confidence in the hazard identification is strengthened primarily by the consistent clinical findings, the clear exposure contrast Nielsen et al. (1988), and the complementary immunologic evidence in Nielsen et al. (1991).

EPA used the LOAEC of 0.4 mg/m^3 (8-hour TWA) from Nielsen et al. (1988) considering the complementary immunologic findings in Nielsen et al. (1991) to derive a candidate POD for effects consistent with respiratory sensitization. EPA did not conduct benchmark dose (BMD) modeling of data from this study to refine the LOAEC because the study only included a single exposure group and was not suitable for modeling, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). EPA applied a UF_H to account for variation in susceptibility within the human population and a LOAEC-to-NOAEC UF (UF_L) to account for uncertainty in extrapolating from a LOAEC to a NOAEC, consistent with EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994) and *A review of the reference dose and reference concentration processes* (U.S. EPA, 2002). Consideration of the magnitude of the UF_H and UF_L is discussed further below.

Intraspecies Uncertainty Factor (UF_H)

To account for variability in susceptibility within the human population, EPA's default approach is to apply a 10× UF to account for intraspecies differences (UF_H) (U.S. EPA, 2002, 1994). The 10× UF_H can be divided into two components: one for toxicodynamic differences (3×) and a second for toxicokinetic differences (3×) (U.S. EPA, 2002, 1994). Several reports discuss advancements in uncertainty analysis, including EPA's *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (U.S. EPA, 2014) and the National Research Council (NRC; now called the National Academies of Science, Engineering, and Medicine [NASEM]) report *Standard Operating Procedures for Developing Acute Exposure Guideline Levels (AEGLs) for Hazardous Chemicals* (NRC, 2001). These reports include considerations for making data-informed refinements to the default UF_H of 10×. EPA's *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (U.S. EPA, 2014) defines intraspecies toxicokinetic variability as differences in tissue concentration attained from the same human external exposure. Specifically, with respect to the intraspecies UF, as noted in the NRC report in Section 2.5.3.3.4, "In those cases in which the mode or mechanism of action is such that the response elicited by exposure to the chemical by different subpopulations is unlikely to differ, an UF_H of 3× is generally used. Typically, this response involves a direct-acting mechanism of toxicity in which metabolic or physiologic differences are unlikely to play a major role."

For input into the draft phthalic anhydride risk evaluation, EPA selected a default factor of 3× to account intraspecies differences in toxicodynamics. A factor of 3× was selected for the toxicodynamics component of the UF_H because very limited data were available to inform the range in variability of the allergic response across the human population associated with exposure to phthalic anhydride. In the Nielsen et al. (1988) study, the IgG (ELISA ratio) in five individuals with asthma (average, 4.6; range, 2.2−7.1) was significantly ($p = 0.005$) greater than the IgG ratio in 26 individuals with no symptoms (average, 1.8; range, 0.6−5.5). From this limited data, the IgG was 2.6 times greater in individuals with

asthma than those with no symptoms. The variability from the lower to upper range of IgG was 3.2 in individuals with asthma as compared to 9.2 in those with no symptoms. These limited data do not support lowering toxicodynamics component of the UF_H below factor of 3×.

- *Option 1: Use of $UF_{H\text{-}TK}$ of 1×:* Option 1 is based on evidence described in Section 4.3.1.1 indicating that molecular initiation event for respiratory sensitization occurs at the point of contact in the respiratory tract. An UF for human toxicokinetic variability is used to account for differences in chemical concentrations at the target tissues, and when the toxic response occurs at the site of contact, at the site of administration, for the same external dose across individuals according to EPA's *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (U.S. EPA, 2014; IPCS, 2005).

  Regional lung exposure is influenced by multiple, interrelated factors. In real-world settings these determinants are correlated (*e.g.,* activity affects breathing rate, and particle size effects depend on breathing rate and activity), making it hard to separate contribution of each factor or to distinguish true interindividual differences, let alone to separate them from measurement-variability. Nonetheless, breathing route and ventilation (behavioral/physiological), together with particle size distribution are the primary determinants of respiratory tract regional exposure (ICRP, 1994). To capture overall human variability, it is important to collectively consider human factors (breathing, ventilation, airway anatomy and physiology) and exposure factors (*e.g.,* activity level) as well as their interactions with particle/aerosol properties (*e.g.,* size distribution).

  EPA used the Multi-Path Particle Dosimetry (MPPD) Model to quantitatively assess the impacts of these determinants across multiple inhalation scenarios—varying human breathing conditions, particle properties, and exposure factors like activity levels in realistic combinations. The MPPD results support an interindividual variability factor of 1.5 to 3.0, which is based on occupational settings as representative of the change in the particle dose rate to the tracheobronchial region with an increase in activity, about 1.5× from light to heavy exercise and about 3× from sitting to heavy exercise (See Appendix E; Section G.3.1.2). Note that MPPD simulations used a baseline of an oronasal mouth breathing during light exercise, representing relatively high delivered dose scenarios reflective of the Nielsen et al. (1988) study.

  As discussed in the EPA's *Review of the Reference Dose and Reference Concentration Processes Document* (U.S. EPA, 2002), the TK component of the intraspecies UF ($UF_{H\text{-}TK}$) is intended to account for variations in susceptibility within the human population due to TK and the possibility that the study population data may not be representative of the dose/exposure-response relationship in the subgroups of the human population. In this case, the Nielsen et al. (1988) study involved highly exposed workers, who may themselves be regarded as in the susceptible population. MPPD simulations indicate that within such highly exposed individuals, target region exposures for the most highly exposed individuals during heavy exercise could be about 1.5-fold higher than typical light exercise conditions and about 3-fold higher than sitting conditions. Moreover, for people with lower exposure than workers, regional lung doses are unlikely to overlap mainly due to interindividual variability, if absent higher exposure levels. Therefore, because the LOAEC is derived from high-exposure worker data and MPPD simulation analysis of interindividual variability based on the exposure conditions reflective of the same study is expected to be at around or below the typically assumed 3 fold, and to align with the NAS recommendation (NRC, 2001), it may be reasonable to reduce the toxicokinetic component of the intraspecies variability ($UF_{H\text{-}TK}$) from the default 3× to 1×.

- *Option 2: Use of UF_H TK of 3×:* Option 2 reflects EPAs application of the ICRP's Human Respiratory Tract Model for Radiological Protection (ICRP, 1994) Nielsen to determine the variability in dose to the tracheobronchial airways that would be expected in a human worker population such as those in the Nielsen et al. (1988) study (described in Appendix E) to inform the $UF_{H\text{-}TK}$. Although heavily exposed workers in the Nielsen et al. (1988) study are an "at-risk" group due to their increased phthalic anhydride exposures relative to workers with minimal exposure, there is also variability in dose to the target tissues in the lower respiratory tract among the heavily exposed workers. An $UF_{H\text{-}TK}$ for human toxicokinetic variability is used to account for differences in chemical concentrations at the target tissues, and when the toxic response occurs at the site of contact, at the site of administration, for the same external dose across individuals according to EPA's *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (U.S. EPA, 2014). Similarly, (NRC, 2001) refers to toxicokinetics as all processes contributing to the concentration and duration of exposure of the active chemical toxicant at the target tissue.

  Variability in the delivered dose of particles to the tracheobronchial region was characterized based on particle penetration through the head using an empirical model developed based on experimental measurements (ICRP, 1994). Central tendency particle penetration through the extrathoracic airways and 95% confidence bounds (*i.e.,* bounds expected to contain 95% of measured data) were estimated for a range of activities levels, particle sizes, and both sexes. The 95% confidence bounds characterize data-derived interindividual variability in particle deposition within the head due to morphometric and physiologic differences among individuals. The ICRP (1994) specifically attributes interindividual variability in laryngeal deposition to "… differences in the morphology and physiology of this region, especially of the larynx and vocal cords." For the aerosols being modeled, the vast majority of particles penetrating the head will deposit in the tracheobronchial region. The results in Appendix E show that, on average, 47% of workers are estimated to have a dose to the tracheobronchial region that is greater than the central tendency estimate. The upper bound for particle deposition in the tracheobronchial region is about 3 times the central tendency estimate regardless of activity level, particle size, or sex of the hypothetical individuals. It was estimated that, on average, 44% of interindividual variability in tracheobronchial deposition is expected to be captured between the central tendency estimate and the 95% upper confidence bound. It was further estimated that a 3 times range around the geometric mean predicted particle deposition within the tracheobronchial region, on average, captures approximately 68% of interindividual variability.

  Collectively, this analysis, which is discussed in greater detail in Appendix E (E.3.1.1), shows that interindividual differences in delivered dose to the tracheobronchial region support the use of a $UF_{H\text{-}TK}$ component of 3× for intraspecies variability. In further support of a $UF_{H\text{-}TK}$ component of 3×, and as discussed above in Option 1, MPPD simulations indicate that within target region exposures for the most highly exposed individuals during heavy exercise could be about 3-fold higher than sitting conditions.

Based on the considerations outlined above, EPA has weighed the strengths and uncertainties associated with Option 1 ($UF_{H\text{-}TK}$ = 1) and Option 2 ($UF_{H\text{-}TK}$ = 3×). For input into the draft risk evaluation of phthalic anhydride, EPA selected Option 2, resulting in a total UF_H of 10× (3× for toxicodynamic and 3× for toxicokinetic components of the UF_H). This conclusion is based on the following:

- *Option 2 is an empirical data derived approach that reflects variability in particle deposition when breathing conditions, activity level, and particle size are held constant:* Option 2 reflects application of dosimetric modeling Nielsen to understand interindividual (intraspecies)

variability that is expected to occur even when factors such as activity level, breathing conditions, and particle size are held constant. It should be noted that MPPD simulations discussed under Option 1 do not estimate changes in particle loss in the extrathoracic airways due to the sex of an individual or changes in upper respiratory tract volume. For the large aerosol (30 µm MMAD) being considered in MPPD simulations for Option 1, particle penetration through the extrathoracic airways adjusted for particle inhalability is approximately equal to deposition within the tracheobronchial airways (see Section F.2.1.1). Therefore, it is critical that simulations accurately predict particle penetration through the upper airways and into the lower respiratory tract. MPPD simulations consider differences in upper respiratory tract volume between males and females with that volume being 50 and 40 mL, respectively. However, changing this volume in the MPPD model from 50 to 40 mL increases the particle transport to the lower respiratory tract but does not increase losses in the extrathoracic region (see Section F3.1.2). Consequently, the MPPD results are inconsistent with the expectation that particle impaction will increase in the extrathoracic airways with a reduction in airway dimensions associated with a reduction in upper respiratory tract volume which in turn reduces particle transport into the tracheobronchial airways.

The ICRP Model that uses a scaling factor to increases particle losses in the head of females relative to males (see Sections F.2.1.1 and F.3.1.1). The MPPD model does not consider upper airway volume much less sex in the calculation of particle deposition efficiency in the extrathoracic region and thus cannot predict interindividual variability for this region of the respiratory tract. To the contrary, Option 2 represents interindividual (intraspecies) variability in particle deposition in the tracheobronchial region due to morphometric and physiologic differences of the extrathoracic airways among individuals, especially of the larynx and vocal cords. These differences support a $UF_{H\text{-}TK}$ component of 3× for intraspecies variability. .

- *Option 1 may underestimate interindividual variability:* Option 1 can provide MPPD model predicted variations in particle deposition to regions of the respiratory tract with changes in factors such as activity level, breathing conditions, and particle size. However, Option 1 does not provide an estimate of interindividual variability around its predicted deposition fractions once the effects of those factors are specified. In short, Option 1 accounts for breathing conditions and particle properties but captures anatomical and physiological variability only to a limited extent. Option 1 shows that when activity level is increased from light to heavy exercise, there is a 1.5 times increase in the dose rate of particles depositing in the tracheobronchial region. This supports a $UF_{H\text{-}TK}$ component of 1 for intraspecies variability. However, because Option 1 evaluates interindividual variability mainly through breathing and activity levels, without fully accounting for anatomical and physiological differences that affect lung dosimetry, it may underestimate the true extent of interindividual variability. It also remains not fully understood whether anatomical/physiological variability influences regional lung exposure in ways that are correlated with, or independent of, the factors emphasized in Option 1. Additionally, MPPD modeling demonstrates that an increase in activity level from sitting to heavy exercise causes a 3 times increase in particles depositing in the tracheobronchial region but the fraction of affected individuals is not known. These uncertainties reduce EPA's confidence in reducing the $UF_{H\text{-}TK}$ component from 3 to 1.

LOAEC-to-NOAEC Uncertainty Factor (UF_L)

EPA applied a LOAEC-to-NOAEC UF (UF_L) to capture the uncertainty associated with the use of a LOAEC as the candidate POD from Nielsen et al. (1988). EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* guidance states to use "up to an additional 10-fold factor when deriving an RfC from a LOAEL instead of a NOAEL" (U.S. EPA, 1994).

EPA's 1994 Guidance Document (U.S. EPA, 1994) and EPA's 2002 review of the reference concentration process (U.S. EPA, 2002) outlines considerations for determining the magnitude of the UF_L, which includes severity of the effect at the LOAEC, slope of the dose-response curve, trend/consistency of effect, functional vs. histopathological evidence, exposure uncertainties, and relationship of the endpoints. Regarding the slope of the dose-response curve, the study does not have a graded dose-response and instead compared a high exposure group (mean 30-minute peak exposure = 6.6 mg/m^3) to a low-exposure group, which was below the limit of detection (*i.e.,* <0.1 mg/m^3). The dataset was not suitable for BMD modeling, consistent with guidance from EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012).

EPA considered these factors in the context of the candidate POD supported by the Nielsen et al. (1988) study. EPA considered two options for the UF_L: Option 1, which reflects a UF_L of 10×, and Option 2, which reflects a UF_L of 3×. Both options for the UF_L are discussed below.

- *Option 1: Use of UF_L of 10×:* Respiratory sensitization is considered a severe outcome (ACGIH, 2025) that would warrant a UF_L of to 10×. The candidate POD from Nielsen et al. (1988) is based on respiratory sensitization reflected by increased prevalence of asthma, conjunctivitis, rhinitis, and increased IgG antibodies. EPA's 1994 Guidance Document (U.S. EPA, 1994) includes phthalic anhydride as an example chemical that elicits an airway immune response of hypersensitivity in humans (Table 2-5 of that guidance). Additionally, serologic sensitization is clinically significant because it indicates immune priming (*i.e.,* a change in immunological status from a non-diseased state to a diseased state) for subsequent allergic responses, the mechanism of which is poorly understood for low-molecular weight sensitizers of the respiratory tract such as phthalic anhydride (Tarlo and Lemiere, 2014).

- *Option 2 Use of UF_L of 3×*: Option 2 considers that a UF_L of 3× is reasonable given the nature and prevalence of outcomes reported.in Nielsen et al. (1988) study; data from Nielsen et al. (1988) do not show a substantial difference between the dose selected as the LOAEC compared to the "slightly" exposed group. Due to understandable practical challenges, the study did not distinguish between sensitized and newly sensitized workers, potentially confounding interpretation of the exposure–response relationship given the two phases of respiratory sensitization—induction and elicitation. The latter are expected to show a greater response magnitude, potentially shifting the dose response curve to the left. Considering the time period of work by the individuals, early exposures and potential induction would have already happened in most individuals (*i.e.,* naïve exposures are expected to be few, if any).

  Nielsen et al. (1988) reported higher prevalences of ocular and nasal symptoms among heavily exposed workers (conjunctivitis 46 vs. 20 %; rhinitis 40 vs. 20 %; asthma 14 vs. 0 %); roughly a two-fold increase for conjunctivitis and rhinitis compared with the "slightly" exposed group; the study by Nielsen et al. did not present between-group significance tests for these symptom proportions, although sufficient data were available for EPA to conduct these statistical analyses (Table_Apx E-1). EPA considers the derivation of a candidate POD based on the totality of evidence consistent with respiratory sensitization from the high-exposure group observations including both ocular/nasal symptoms and immunological markers in the Nielsen et al. (1988) study. However, EPA also notes that the observed symptom prevalences are not far from current day general population benchmarks in the United States (*e.g.,* ≈8% adult asthma (CDC, 2026) and ≈26% seasonal allergy (primarily including allergic rhinitis) in adults (Ng and Boersma, 2023)); however, it is notable that the background incidence of asthma was 0% in the control group in the study by Nielsen et al. (1988). EPA acknowledges that the Nielsen et al. (1988) study was conducted in Sweden in the 1980s. Therefore, a more appropriate comparator would

be to Sweden general population benchmarks from the 1980s; however, asthma benchmarks in Sweden during this time are less well understood. One study suggests that active asthma rates in Sweden were low (1.3–2.3%) in the 1960s (Bjornsson et al., 1994), while another study reported levels of active asthma to be around 9.1% in Sweden in 2016 (Borna et al., 2019), which is similar to the current U.S. benchmark. It is unclear whether the five cases of asthma in the Nielsen et al. (1988) are work-related exacerbation of existing asthma or incidents of work-related new onset asthma. Considering these points, the findings of the Nielsen et al. (1988) study data support the UF_L of 3×.

Based on the considerations outlined above, EPA has weighed the strengths and uncertainties associated with Option 1 (UF_L = 1×) and Option 2 (UF_L = 3×). For input into the draft risk evaluation of phthalic anhydride, EPA selected Option 2 (UF_L of 3×) based on uncertainties of the severity of the effect observed in the epidemiological study by Nielsen et al. This conclusion is based on the following:

- *Limited Available Information on the Prevalence of Asthma:* Respiratory sensitization is generally considered a severe outcome (ACGIH, 2025). However, the candidate POD from Nielsen et al. (1988) is based on respiratory sensitization reflected by increased prevalence of asthma, conjunctivitis, rhinitis, and increased IgG antibodies in a high exposure group compared to a low exposure group, and the magnitude these increases does not support a UF_L of 10. Observed symptom prevalences for asthma in the highly exposed worker group (14%) are not far from current day general population benchmarks in the United States (*e.g.,* ≈8% adult asthma (CDC, 2026) and ≈26% seasonal allergy (primarily including allergic rhinitis) in adults (Ng and Boersma, 2023)). These relative prevalences support a UF_L of 3×.

Overall, for its draft risk evaluation of phthalic anhydride, EPA selected a total UF of 30× for use as the benchmark MOE based on a UF_H of 10× to account for human variability, and a UF_L of 3× to account for extrapolating from a LOAEC to a NOAEC.

4.3.1.3.2 Alternative POD from Animal Evidence – Phthalic Anhydride

EPA identified six studies of animals exposed to phthalic anhydride via inhalation (Table 4-18). Four of the available studies only evaluated a single inhalation exposure concentration of phthalic anhydride and are therefore not suitable for dose-response assessment and were not considered further (De Jong et al., 2009; Arts et al., 2008; IIT Research Institute, 1996; Blaikie et al., 1995; IIT Research Institute, 1995), although the study by Blaikie et al. (1995) provided information on the potential for cross-route sensitization of phthalic anhydride (*i.e.,* dermal induction and inhalation challenge).

Of the available studies with more than one dose level, two adhere to an induction and challenge paradigm to induce respiratory sensitization in which animals were exposed via inhalation to phthalic anhydride for 5 consecutive days (induction), rested for two to three weeks, and then challenged with a single inhalation exposure to phthalic anhydride (Sarlo et al., 1994; Sarlo and Clark, 1992). Sarlo et al. (1992) exposed female Harley guinea pigs to two concentrations of phthalic anhydride dust (0.05–0.2, or 0.6–6 mg/m^3) for 3 hours per day for 5 days, rested animals for 5 days, and then challenged them with a single 30-minute inhalation exposure to GPSA-phthalic anhydride conjugate. Upon challenge, only the high exposure group exhibited immediate onset respiratory reactions and increased antibody levels. No changes were observed in the lower dose group. Limitations of this study included exposure characterization issues; exposure concentrations could not be stably maintained, resulting in exposure levels that varied by up to an order of magnitude. Therefore, this study was not further considered for dose-response assessment or for deriving a candidate inhalation POD.

Another study by Sarlo et al. (1994) provided dose-response information from available studies of animals exposed to phthalic anhydride. Briefly, female guinea pigs were exposed to 0, 0.5, 1, or 5 mg/m^3 phthalic anhydride dust for 3 hours per day for 5 consecutive days and were then challenged with a single 30-minute exposure to 5 mg/m^3 phthalic anhydride dust 2 weeks later. Measured chamber concentrations were: 0.55, 1.27, 5.57 mg/m^3; MMAD (± GSD) = 3.12 µm ± 2.02, 3.26 µm ± 1.96, and 3.91 µm ± 2.08. Dose-dependent increases in serum IgG antibody levels were observed (18 hours prior to challenge) at 0.55 mg/m^3 and above in a dose-dependent manner, supporting a LOAEC of 0.55 mg/m^3 (no NOAEC established). This LOAEC of 0.55 mg/m^3 (550 µg/m^3) from Sarlo et al. (1994) was further considered as an alternative candidate inhalation POD.

In order to compare this value to one relevant for an 8-hour TWA for exposed worker populations (Section 4.3.1.3.1), EPA calculated a duration adjusted LOAEC as shown in Equation 4-1 for Sarlo et al. (1994).

Equation 4-1. Duration Adjusted LOAEC for Sarlo et al. (1994)

$$206.3\frac{\mu g}{m^3} = 550\frac{\mu g}{m^3} \times \frac{3\ hr}{8\ hr}$$

The duration-adjusted LOAEC is 0.2063 mg/m^3 (or 206.3 µg/m^3) for an 8-hour TWA. A total UF of 300 was selected for use as the benchmark MOE (based on a UF_A of 3× to account for interspecies variability; a UF_H of 10× to account for intraspecies variability; and a UF_L of 10× to account for the lack of NOAEC in the critical study). EPA reduced the UF_A from 10× to 3× through dosimetric modeling using the Regional Deposited Dose Ratio (RDDR) model (U.S. EPA, 1994) to derive a HEC, which reduced the toxicokinetic component of the UF_A from 3× to 1×, resulting in a UF_A of 3× to account for remaining toxicodynamic variability. EPA applied the RDDR Model discussed in Appendix E. Briefly, EPA calculated HECs based on the exposure conditions expected from loading a reactor (MMAD of 30 µm; light activity; oronasal breathing) for an 8-hour TWA to be 0.099 mg/m^3 (99 µg/m^3), which reflects worker exposure concentrations producing tracheobronchial surface doses equivalent those in guinea pigs exposed to 0.55 mg/m^3 for 3 hours/day.

EPA also considered refining the UF_L given the combined data from the two studies by Sarlo et al. (1992) and Sarlo et al. (1994). Sarlo and Clark (1992) did not report detectable levels of IgG antibodies at their lowest tested concentration (reported as a range of 0.05−0.2 mg/m^3). Assuming the true NOAEC for respiratory effects is closer to 0.05 mg/m^3 than 0.2 mg/m^3, these data support retaining the full UF_L of 10× given the order of magnitude difference between the LOAEC in Sarlo et al. (Sarlo et al., 1994) is 0.5 mg/m^3 and the NOAEC in Sarlo and Clark (1992) of 0.05 mg/m^3 based on a ratio of the LOAEC to the NOAEC. Alternatively, assuming the midpoint of the reported range (*i.e.,* 0.125 mg/m^3) supports a UF_L of 4×. Given the uncertainty in how informative the ratio of the doses at the LOAEC and the NOAEC are in this case, a UF_L of 10× is consistent with EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* guidance, which states to use "up to an additional 10-fold factor when deriving an RfC from a LOAEL instead of a NOAEL" (U.S. EPA, 1994).

Study details of candidate POD and uncertainties associated with the candidate POD from Sarlo et al. (1994) are summarized in Table 4-20.

4.3.1.3.3 Alternative POD from Animal Evidence - TMA

As discussed in Appendix B, EPA conducted a read-across assessment to identify suitable data from an analog, TMA. EPA identified TMA as the most suitable analog for phthalic anhydride based on similarities in physical and chemical as well as fate properties, toxicity (*i.e.,* respiratory sensitizer), and chemical structure. EPA also identified several reasonably available inhalation dose-response studies of TMA, including a 2-week inhalation study of male and female SD rats exposed to 0, 10, 30, 100, and 300 µg/m^3 TMA for 6 hours per day, 5 days per week for 2 weeks (Leach et al., 1987; Zeiss et al., 1987) and a 13-week study of male and female SD rats, which included a 6.5-week interim sacrifice, exposed to 0, 2, 15, and 50 µg/m^3 TMA for 6 hours per day, 5 days per week for 13-weeks (measured concentrations: 2.2, 15.4, 53.5 µg/m^3) (Leach et al., 1989; IIT Research Institute, 1988). Across available studies, no NOAEC could be established. Dose-related increases in TMA-specific antibodies were observed in all exposure groups, supporting a LOAEC of 10 µg/m^3 in the 2-week study, and a LOAEC of 2.2 µg/m^3 in the 6.5- and 13-week studies (no NOAEC identified). The LOAEC from Leach et al. (1989; IIT Research Institute, 1988) of 0.0022 mg/m^3 (2.2 µg/m^3) was further considered as an alternative candidate POD.

In order to compare this value to the 8-hour TWA vales for phthalic anhydride in exposed worker populations (Section 4.3.1.3.1) and guinea pigs (Section 4.3.1.3.2), EPA calculated a duration adjusted LOAEC as shown in Equation 4-2 for Leach et al. (1989).

Equation 4-2. Duration Adjusted LOAEC for Leach et al. (1989)

$$1.65\,\frac{\mu g}{m^3} = 2.2\,\frac{\mu g}{m^3} \times \frac{6\ hr}{8\ hr}$$

The duration-adjusted candidate POD is 0.00165 mg/m^3 (or 1.65 µg/m^3) for an 8-hour TWA. A total UF of 300 was selected for use as the benchmark MOE (based on a UF_A of 3× to account for interspecies variability; a UF_H of 10× to account for intraspecies variability; and a UF_L of 10× to account for the lack of NOAEC in the critical study). The UF_A was reduced from 10× to 3× following modeled dosimetry refinements using the MPPD Model (version 3.0.4) as described in Appendix E. Briefly, EPA estimated the daily dose rate of TMA particles, specifically the deposited dose per unit surface area of the tracheobronchial region. The equivalent cross-species exposure concentrations (in µg/day TMA per cm^2 of surface area) were estimated using the exposure period of 0.5 hours per workday in humans from Nielsen et al. (1988). EPA then determined equivalent exposure concentrations that rats (average of males and females) would need to be exposed to for 6 hours to produce the estimated daily dose rate in workers (average of males and females) assuming aerosol sizes of 30, 50, and 60 µm MMAD (MMAD was not reported in Nielsen and colleagues). The human equivalent exposure concentration were estimated for workers having exposures of 30 minutes/workday to varied aerosols (MMAD of 30, 50, and 60 µm) to match the $N\dot{D}_{TB}$ estimated for rats exposed to 2.2 µg/m^3 of aerosol (1.74 µm MMAD, 1.42 GSD) with exposures of 6 hours/day and 5 days/week was also estimated. EPA calculated HECs based on the exposure conditions expected from loading a reactor (MMAD of 30 µm; light activity; oronasal breathing) for an 8-hour TWA to be 0.03 mg/m^3 (30 µg/m^3) which reflects worker exposure concentrations producing tracheobronchial surface doses equivalent those in rats exposed to 0.0022 mg/m^3 for 6 hours/day. Therefore, EPA was able to refine the UF_A TK component from 3× to 1×, resulting in a UF_A of 3×.

Study details of candidate POD of 0.03 mg/m^3 and uncertainties associated with the candidate POD from Leach et al. (1989) are summarized below in Table 4-20.

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Brief Study Description	Candidate POD (mg/m^3)	Critical Effect	Duration Adjusted POD (HEC) for an 8-Hour TWA (mg/m^3) [a]	UFs	Effective Hazard Value (mg/m^3) [b]	TSCA Study Quality Rating
Epidemiological study of factory workers in Sweden. Workers were classified as "heavily" (n = 28 reactor loaders; n = 7 repair men) or "slightly" (n = 25) exposed based on their job. Mean peak air concentrations were 6.6 mg/m^3 in heavily exposed workers, equivalent to an 8-hour TWA of 0.4 mg/m^3 (Nielsen et al., 1988)	LOAEC = 0.4 (8-hour TWA)	↑ incidence of asthma, conjunctivitis, and rhinitis, as well as ↑serum specific IgG antibodies	0.4	UF_H= 10 UF_L = 3 *Total UF = 30*	0.0133	Low-Medium
Female Hartley guinea pigs (8–16/group) exposed via whole-body inhalation to 0 (filtered air), 0.5, 1, or 5 mg/m^3 phthalic anhydride dust for 3 h/day, 5 consecutive days (measured concentrations: 0.55, 1.27, 5.57 mg/m3; MMAD (± GSD) = 3.12 µm ± 2.02, 3.26 µm ± 1.96, 3.91 µm ± 2.08). Two weeks after the last exposure, control and high-dose guinea pigs challenged with 5 mg/m^3 phthalic anhydride dust for 30 minutes, while animals in all treatment groups were challenged with 2 mg/m^3 aerosolized phthalic anhydride-GPSA conjugate for 30 minutes (Sarlo et al., 1994)	LOAEC = 0.55 (3-hour TWA)	↑ serum IgG antibodies in Guinea pigs after induction	0.099	UF_H = 10 UF_A= 3 UF_L = 10 *Total UF = 300*	0.00033	Medium
Male SD rats (10/dose) exposed to target concentrations of 0, 2, 15, 50 µg/m3 TMA (measured: 2.2, 15.4, 53.5 $µg/m^3$) for 6 hours/day, 5 days/week for 6.5 or 10 weeks (MMAD ± GSD: 1.7 µm ± 1.4; 2.2 µm ± 1.4; 2.2 µm ± 1.4) (Leach et al., 1989; IIT Research Institute, 1988)	LOAEC = 0.0022 (6-hour TWA)	↑ serum IgG antibodies specific to TMA-RSA	0.030	UF_H = 10 UF_A= 3 UF_L = 10 *Total UF = 300*	0.0001	(NA) [d]

POD = Point of departure; TWA = time weighted average; LOAEC = lowest-observable-adverse-effect-concentration; MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation; PA-GPSA = phthalic anhydride conjugated to guinea pig serum albumin; UF = Uncertainty factor; UFA = Interspecies UF; UFH= Intraspecies UF; UFL= LOAEL-to-NOAEL UF

[a] HECs for duration adjusted PODs for an 8-hour TWA for Sarlo et al. (1994) and Leach et al. (1989) were calculated as described in Appendix E based on the exposure conditions that would be expected from human workers loading a reactor.

[b] Effective hazard value is the POD divided by the UF

[c] The study quality rating for Nielsen et al. (1988) of "Low-Medium" reflects the rating of "Low" for "irritation of the upper airways, rhinitis, asthma, phthalic anhydride-induced asthma, chronic productive bronchitis, spirometry" and "Medium" for serum specific IgG antibodies.

[d] No evaluation was conducted for Leach et al. EPA ultimately did not select this candidate POD for use in quantitative risk characterization, therefore did not conduct a data quality evaluation for this study.

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4.3.1.3.4 Points of Departure from Other Assessments

EPA evaluated existing hazard values from other organizations, including from regulatory and authoritative agencies, as well as one stakeholder. Existing inhalation hazard values for phthalic anhydride, including OELs, span nearly four orders of magnitude and range from 0.002 to 12 mg/m^3 (*i.e.,* the ACGIH 8-hour TWA threshold limit value [TLV] of 0.002 mg/m^3 and the Occupational Safety and Health Administration [OSHA] 8-hour TWA permissible exposure limit [PEL] of 12 mg/m^3). Other organizations including National Institute for Occupational Safety and Health (NIOSH), California OEHHA, Health Canada, and Exxon Mobil Biosciences, have also derived inhalation hazard values, which are summarized below in Table 4-21. However, the EU and the Netherlands have determined that available data for phthalic anhydride are not suitable to dose-response assessment and refrained from deriving inhalation hazard values for phthalic anhydride (Table 4-21).

The current OSHA 8-hour TWA PEL of 12 mg/m^3 was originally promulgated in 1971[5], and the NIOSH recommended exposure limit (REL) of 6 mg/m^3 (8-hour TWA) was derived in 1988. In the Federal Register Notice (FRN) for the final rule, OSHA indicated the PEL reflects the level at which risk of respiratory irritation and skin and respiratory sensitization will be reduced (see FRN 54 No 12, page 2639–2640). EPA could not obtain additional documentation regarding the POD used to set the OSHA PEL of 12 mg/m^3 and NIOSH REL of 6 mg/m^3.

ExxonMobil Biomedical Sciences (2025) submitted information to EPA regarding the derivation of their internal OEL of 0.05 mg/m^3. The OEL was derived from the aforementioned study by Sarlo et al. (1994) from a LOAEC of 0.5 mg/m^3 based on respiratory sensitization (increased IgG levels before challenge). Due to differences in assumptions between EPA and Exxon regarding application of UFs applied for the LOAEC of 0.5 mg/m^3 from Sarlo et al. (1994), the effective hazard value (*i.e.,* the POD divided by the total UF) from the candidate POD from Sarlo et al. differed.

Similar to EPA's proposed POD, Health Canada (2019) and California OEHHA (2008) both used the occupational exposure study of workers by Nielsen et al. (1988) to derive inhalation hazard values. In its screening-level risk assessment of phthalic anhydride, Health Canada (2019) selected the 30-minute peak concentration of 6.6 mg/m^3 and 8-hour TWA of 0.4 mg/m^3 from the occupational study by Nielsen et al. (1988) to characterize risk from inhalation exposures to phthalic anhydride based on effects consistent with respiratory sensitization. Health Canada did not explicitly report the UFs selected for use as their benchmark, however, MOEs calculated by Health Canada range from 308 (spray paint inhalation exposure scenario) to 9,677 (indoor air exposure scenario), and Health Canada concluded phthalic anhydride does not or may not constitute a danger in Canada to human life of health.

Similarly, California OEHHA (2008) extrapolated a chronic reference exposure level of 0.02 mg/m^3 from the occupational studies reported by Nielsen et al. (1991; 1988). The critical effect that served as the basis for the chronic reference exposure level was "eye and respiratory irritation, asthma, and bronchitis in occupationally exposed workers." To derive the chronic reference exposure level, OEHHA reported that the mean peak exposure concentration was adjusted to an equivalent continuous exposure of 2.3 mg/m^3 assuming a daily air intake of 20 m^3 compared to 10 m^3 during a workday. California

[5] OSHA adopted the ACGIH TLV for phthalic anhydride of 1 ppm (6.1 mg/m^3) as the PEL in 1989, concluding that the PEL will "reduce the significant risk of respiratory irritation and skin and pulmonary sensitization." This action reduced the PEL from 2 ppm (12 mg/m^3); however, the rule was vacated in 1992 and the prior PEL reinstated. OSHA (FRN 54 No. 12).

OEHHA selected a UF_L of 10× and a UF_H of 10×, which resulted in a chronic reference exposure level of 0.02 mg/m^3 (Table 4-21).

In 2017, ACGIH (2025) derived an 8-TWA TLV for phthalic anhydride of 2 µg/m^3 (or 0.002 mg/m^3) for the inhalable fraction and vapor based on respiratory sensitization as the critical effect. In setting this value, ACGIH considered effect levels for respiratory sensitization from experimental animal and human epidemiology studies and selected a TLV below the range of observed effect levels; however, EPA was unable to obtain sufficient documentation to clarify key aspects of the derivation. Specifically, ACGIH does not clearly define the POD used to mark the beginning of the extrapolation to their TLV, and ACGIH does not clearly define the factors used to derive and set their TLV, including use of UFs typically considered in EPA assessments. The TLV is intended to prevent new cases of respiratory sensitization but may not protect already sensitized workers; as ACGIH states, "The recommended TLV may not necessarily protect against an allergic reaction in previously sensitized individuals; accordingly, exposures should be kept as low as possible below the recommended TLV" (ACGIH, 2025).

Assessments from other groups have refrained from deriving a quantitative inhalation hazard value for occupational exposures, citing the uncertainties with respiratory sensitizers (EC, 2011) or limitations in available studies (DECOS, 2010) that preclude the ability to define a POD. The European Commission's Scientific Committee on Occupational Exposure Limits (SCOEL) concluded that human dose-response data for phthalic anhydride is poor and that available human epidemiologic data do not support identification of a NOAEC or a LOEAC for respiratory sensitization (EC, 2011). Although SCOEL recommended that a short-term exposure limit (STEL) for phthalic anhydride would be advisable because peak exposures are likely to be important to induction of sensitization, "a scientifically based value is impossible to assess based on available data." The Dutch Expert Committee on Occupational Standards (DECOS) did not derive a hazard value for use in quantitative risk characterization due to the lack of adequate data on respiratory sensitization and respiratory symptoms associated with occupational exposures to phthalic anhydride (DECOS, 2010).

Table 4-21. Occupational Exposure Limits and Other Inhalation Hazard Values for Phthalic Anhydride Set by Various Organizations

Administrative Agency or Other Authoritative Source and Reference	Inhalation Hazard Value (for 8-Hour TWA) (mg/m^3)	POD, Key Study, and Details
OELs for phthalic anhydride set by various organizations		
ACGIH *TLV: Phthalic Anhydride* (ACGIH, 2025)	0.002	The critical effect was respiratory sensitization. EPA could not obtain additional documentation that clarified the derivation of the POD supported by the critical effect(s) or UFs applied to the POD. However, ACGIH notes that the TLV is intended to be protective of new cases of respiratory sensitization, but is not intended to be protective of existing cases.
California OEHHA *Appendix D.3 Chronic RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines* (CalEPA, 2008)	0.02	The critical effect was "eye and respiratory irritation, asthma, and bronchitis in occupationally exposed workers" The chronic reference exposure level was derived from (Nielsen et al., 1991; Nielsen et al., 1988). The mean peak exposure concentration (6.5 mg/m^3) was adjusted to an equivalent continuous exposure of 2.3 mg/m^3. Use of the UF_L of 10× and UF_H of 10× resulted in an estimated chronic reference exposure level of 0.02 mg/m^3

Administrative Agency or Other Authoritative Source and Reference	Inhalation Hazard Value (for 8-Hour TWA) (mg/m^3)	POD, Key Study, and Details
Exxon Mobil (Nyambego, 2025)	0.05	The OEL was derived from Sarlo et al. (1994) from a LOAEL of 0.5 mg/m^3 based on respiratory sensitization (increased IgG levels). A total UF of 10× was applied.
NIOSH REL Appendix G: 1989 Air Contaminants Update Project – Exposure Limits NOT in Effect	6	EPA could not obtain documentation.[b]
OSHA PEL 29 CFR 1910.1000	12	OSHA adopted the ACGIH TLV for phthalic anhdyride of 1 ppm (6.1 mg/m^3) as the PEL in 1989, concluding that the PEL will "reduce the significant risk of respiratory irritation and skin and pulmonary sensitization." This action reduced the PEL from 2 ppm (12 mg/m3); however, the rule was vacated in 1992 and the prior PEL reinstated. OSHA (FRN 54 No. 12). In the FRN for the final rule, OSHA indicated the PEL reflects the level at which risk of respiratory irritation and skin and respiratory sensitization will be reduced (see pp. 2639–2640). EPA could not obtain additional documentation or information on the PEL. [b]
European Commission's Scientific Committee on Occupational Exposure Limits (2011)	NA	Concluded that human dose-response data for phthalic anhydride is poor and that available human epidemiologic data do not support identification of a NOAEL or a LOAEL for respiratory sensitization.
Dutch Expert Committee on Occupational Safety (DECOS) (2010)	NA	Work-related respiratory symptoms reported at average full-day exposure to 0.4 mg/m^3 combined with peak exposures to up to 13 mg/m^3 (Nielsen et al., 1991; Nielsen et al., 1988). DECOS "considers the available data not suitable for deriving a health-based recommended OEL or reference value. In conclusion, the committee abstains from giving a recommendation."
Other inhalation hazard values for phthalic anhydride set by various organizations		
Health Canada *Screening Assessment Carboxylic Acid Anhydrides Group* (Health Canada, 2019)	0.4 [b]	Effects consistent with respiratory sensitization. Discontinuous occupational study of 23 resin plant workers exposed to a mean peak concentration of 6.6 mg/m^3 phthalic anhydride; 8-hour TWA of 0.4 mg/m^3 over one day (Nielsen et al., 1991; Nielsen et al., 1988) MOEs ranged from 308 (spray paint inhalation exposure scenario) to 9,677 (indoor air exposure scenario)

ACGIH = American Conference of Governmental Industrial Hygienists; POD = point of departure; OEL = occupational exposure limit; TLV = threshold limit value; PEL = permissible exposure limit; REL = recommended exposure limit; NIOSH =National Institute of Occupational Safety and Health; OSHA = Occupational Safety and Health Administration; OEHHA = Office of Environmental Health Hazard Assessment; NA = not available

[a] Although EPA could not obtain documentation, it is likely that the OSHA PEL and NIOSH REL are based on the outdated ACGIH TLV from 1968, supported from studies by Baader (1955) and Menschick (1955).

[b] Health Canada (2019) did not report application of UFs with which to compare to their MOEs; EPA assumed a value of 100 in order to represent this assessment for ease of comparison across existing hazard values.

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Figure 4-4 represents EPAs duration-adjusted proposed PODs (purple bar) in the context of the existing inhalation hazard values from other organizations (represented as grey bars).

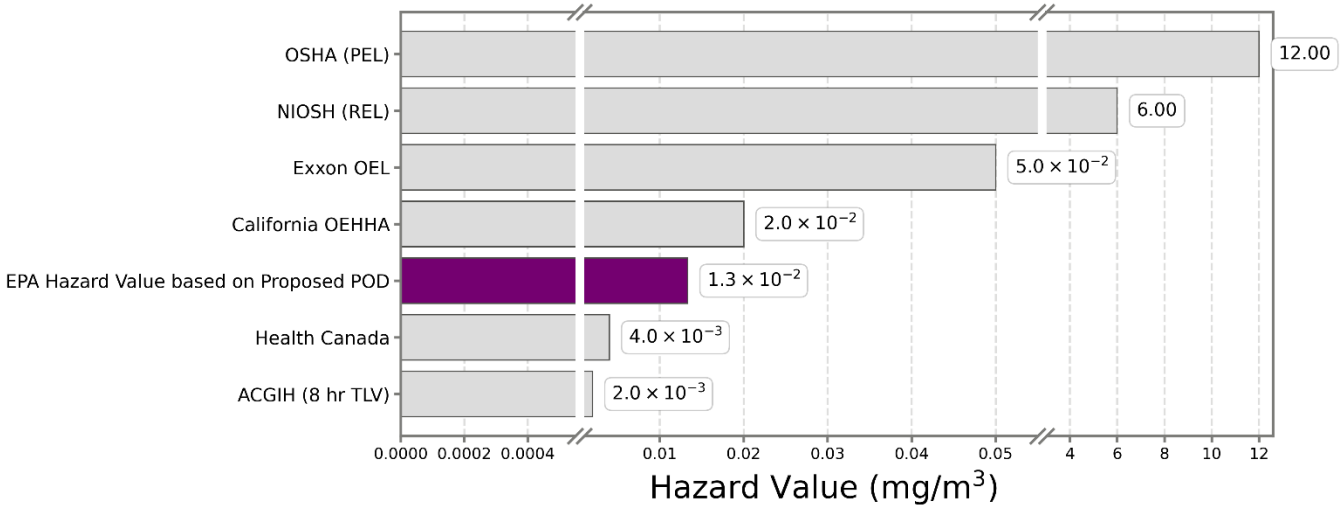

Figure 4-4. Overview of Existing Inhalation Hazard Values from Various Organizations[a] [b]

ACGIH = American Conference of Governmental Industrial Hygienists; OEL = occupational exposure limit; TLV = threshold limit value; PEL = permissible exposure limit; REL = recommended exposure limit; NIOSH =National Institute of Occupational Safety and Health; OSHA = Occupational Safety and Health Administration; OEHHA = Office of Environmental Health Hazard Assessment

Purple bar shows LOAEC (effective hazard values) for EPAs proposed POD described in Section 4.3.1.3.1 (*i.e.,* the human study by Nielsen et al., 1988) (*i.e.,* the POD divided by the Total UF).

[a] Due to the large range in existing values, the x-axis is broken to enhance visibility.

[b] Health Canada did not report application of UFs with which to compare to their MOEs; EPA assumed a value of 100 in order to represent this assessment for ease of comparison across existing hazard values.

The current OSHA 8-hour TWA PEL of 12 mg/m^3 was originally promulgated in 1971[6], and the NIOSH REL of 6 mg/m^3 was derived in 1988. Studies that have been published since the OSHA and NIOSH values (Sarlo et al., 1994; Nielsen et al., 1991; Nielsen et al., 1988) indicate that these values are too high and not health protective. For example, the Nielsen et al. (1988) epidemiology study supports a LOAEC of 0.4 mg/m^3 (8-hour TWA), which shows a similar value to the LOAEC of 0.1875 mg/m^3 (8-hour TWA) supported by the Sarlo et al. (1994) guinea pig study. These LOAECs for respiratory sensitization are well-below the OSHA PEL and NIOSH REL.

The OEL of 50 µg/m^3 derived by ExxonMobil Biomedical Sciences (2025) is based on a LOAEC of 0.5 mg/m^3 from Sarlo et al. (1994). However, EPA considered there to be substantial differences in animals and humans in terms of respiratory tract anatomy as well as uncertainties associated with respiratory sensitization in general, as discussed above in Sections 4.3.1.3.2 and 4.3.1.3.3, and reflected by the total UFs shown in Table 4-20. The candidate POD from Sarlo et al. (1994) and UFs (total UF = 300) would yield an effective hazard value of 0.03 µg/m^3 (0.00033 mg/m^3), which is an order of magnitude below the ACGIH value (0.002 mg/m^3).

[6] OSHA adopted the ACGIH TLV for phthalic anhydride of 1 ppm (6.1 mg/m^3) as the PEL in 1989, concluding that the PEL will "reduce the significant risk of respiratory irritation and skin and pulmonary sensitization." This action reduced the PEL from 2 ppm (12 mg/m^3); however, the rule was vacated in 1992 and the prior PEL reinstated. OSHA (FRN 54 No. 12).

Ultimately, EPA is proposing the candidate POD from the occupational study by Nielsen et al. (1988) based on a LOAEC of 0.4 mg/m^3 (8-hour TWA; effective hazard value [*i.e.,* POD/ Total UF] = 1.3 × 10^{-2} mg/m^3). The study by Nielsen et al. is a human study in exposed workers and EPA preferred to rely on human data over the animal data for the POD based on respiratory sensitization because there is more uncertainty related to extrapolation of animal data for respiratory sensitization endpoints. The study by Nielsen et al. has also been used quantitatively in risk assessments by Health Canada (2019) and California OEHHA (CalEPA, 2008).

4.3.1.4 Weight of Scientific Evidence Conclusions: Inhalation POD

EPA considered the weight of scientific evidence including reasonably available studies discussed in the context of AOP 39, including *in chemico*, *in vitro*, and *in vivo* studies in animals and of humans, as well as existing OELs of phthalic anhydride. EPA has preliminarily selected a POD of 0.4 mg/m^3 which is based on increased incidence of respiratory symptoms (*e.g.,* increased incidence of asthma, conjunctivitis, rhinitis) and increased serum specific IgG antibodies consistent with respiratory sensitization in workers exposed to phthalic anhydride (Nielsen et al., 1988). A total UF of 30 was selected for use as the benchmark MOE (based on a UF_H of 10× and a UF_L of 3×). The draft POD is supported by the following weight of scientific evidence considerations:

- There is consistent, strong, coherent, and biologically plausible evidence for respiratory sensitization in workers exposed to phthalic anhydride via inhalation.
- Phthalic anhydride tested positive for respiratory sensitization in a variety of *in chemico*, *in vitro*, and *in vivo* assays across KEs in AOP 39 (Section 4.3.1.1). Phthalic anhydride tested positive for sensitization in two DPRAs and one kDPRA (KE 1; Section 4.3.1.1.1); positive for DC activation in one GARDair assay and one ALISens assay (KE 3; Section 4.3.1.1.3); one respiratory LLNA (KE 4; Section 4.3.1.1.4). The combined human and animal evidence support that phthalic anhydride elicits an allergic respiratory hypersensitivity response and is a respiratory sensitizer (Section 4.3.1.1.5).
- EPA identified one ALIsens assay that provided relevant data for KE 2 (Section 4.3.1.1.2). Although increases in pro-inflammatory cytokines were observed, EPA considered the result "inconclusive" for KE 2 because there is not yet a data-interpretation procedure for this assay for KE 2 to clarify the biological significance of the observed magnitudes of changes (≈2-fold) across various cytokines as well as the patterns of changes that reflect a positive result.
- Phthalic anhydride tested positive in one GARDair assay (NTP, 2026) and one ALIsens assay, but negative in the second ALIsens assay (Section 4.3.1.1.3).
    - EPA notes that phthalic anhydride tested positive for this KE for the respiratory sensitization AOP, but not for the analogous dermal KE in the dermal AOP. One explanation may be that the test conditions in the ALIsens assay, which involve an air-liquid interface, would not favor hydrolysis of phthalic anhydride to *o*-phthalic acid, a non-sensitizer, while the aqueous conditions of the *in vitro* assays available for KE 3 in the skin sensitization AOP would favor hydrolysis.
- EPA has robust confidence that respiratory sensitization is the most sensitive adverse health outcome for the inhalation route of exposure based on coherence across epidemiological, animal toxicology, and mechanistic streams of evidence (Section 4.3.1.2) including:
    - Of the six epidemiological studies, four (two Medium; two Low confidence) found consistent evidence of work-related asthma or respiratory symptoms, including immediate, late, or dual type asthmatic reactions, which in some cases coincided with increased serum levels of specific IgG against phthalic anhydride-HSA (Nielsen et al.,

1991; Nielsen et al., 1988) (outcome not evaluated in 3 remaining studies). Across the targeted phthalic anhydride plant studies, brief peak dust exposures during reactor loading appear more critical than low full-shift averages (Nielsen et al., 1991; Nielsen et al., 1988; Wernfors et al., 1986). Wernfors et al. (1986) provides strong SIC evidence (including elicitation at 0.5 mg/m^3 for 10 minutes), while Barker et al.(1998) is supportive for acid anhydrides in general but is not specific to phthalic anhydride. Nielsen et al. (1991) reports higher prevalences of symptoms and elevated phthalic anhydride-specific IgG but is less informative for defining a POD than Nielsen et al. (1988). The TOMA screening studies lacked phthalic anhydride-specific endpoints and exposure data (TOMA, 1982, 1981, 1979).

- Six *in vivo* experimental animal toxicology studies demonstrate consistent evidence of respiratory sensitization across species (*i.e.,* rats, guinea pigs, and mice)
  - Two studies demonstrate changes in lung function and/or increases breathing rate in guinea pigs that correspond with increased IgG antibody levels or serum-specific IgG antibody levels (Sarlo et al., 1994; Sarlo and Clark, 1992).
  - Other studies demonstrate increases in lymphocyte proliferation in mandibular lymph nodes in mice (De Jong et al., 2009; Arts et al., 2008) and incidence of lung histopathology (*e.g.,* hemorrhagic foci) in 1 study of each rats, mice, and guinea pigs (De Jong et al., 2009; Arts et al., 2008; IIT Research Institute, 1996; Sarlo et al., 1994)
- Mechanistic evidence from two of three *in vitro* assays which indicate that phthalic anhydride is a respiratory sensitizer.
  - Phthalic anhydride is positive in the GARDair assay (NTP, 2026) and ALIsens model (Invitrolize, 2024)
- TMA analog data (Appendix B) further support the conclusion that phthalic anhydride is a respiratory sensitizer.
  - Consistent evidence of work-related asthma in human occupational exposure studies, three of which provide evidence to inform an exposure gradient for TMA exposure and respiratory and/or immunogenic outcomes (*i.e.,* TMA-HSA antibody levels) (Grammer et al., 1999; Barker et al., 1998; Grammer et al., 1992).
  - Coherent evidence in six *in vivo* animal toxicology studies, including consistent increases in relative lung weight and/or volume; incidence of gross and microscopic lung findings (*e.g.,* hemorrhagic lung foci, multifocal lobular bronchopneumonia); and serum antibody levels against TMA.

- In the key study used to derive the POD, phthalic anhydride elicited adverse symptoms consistent with respiratory sensitization (*i.e.,* increased incidence of asthma, conjunctivitis, and rhinitis), together with increased serum IgG antibodies specific to phthalic anhydride-HSA at an 8-hour TWA of 0.4 mg/m^3 in an occupational setting (Nielsen et al., 1988). Brief peak exposures during reactor loading averaged 6.6 mg/m^3 (range: 1.5–17.4 mg/m^3) for 5 to 30 minutes, with irregular respirator use and potential co-exposures to other anhydrides noted. Similarly, respiratory sensitization has been observed in guinea pigs at doses as low as 0.55 mg/m^3 (equivalent to an 8-hour TWA of 0.2063 mg/m^3) (Sarlo et al., 1994).
- Existing assessments have also consistently concluded that phthalic anhydride is a respiratory sensitizer, including California OEHHA (2008), the OECD (2005), Health Canada (2019), Australia NICNAS (2013), and ACGIH (2025). California OEHHA (2008) and Health Canada

(2019) have also selected the same POD of 0.4 mg/m^3 from Nielsen et al. (1988) for use in risk characterization or for deriving a chronic reference exposure level.

- Although EPA has robust confidence that phthalic anhydride is a respiratory sensitizer based on the weight of scientific evidence, there remain uncertainties associated with the use of respiratory sensitization outcomes in a regulatory context—particularly with animal models. This is due to the lack of validated *in vivo* models of respiratory sensitization and species differences in respiratory and immunological mechanisms between humans and animals. Consequently, EPA favored the human study by Nielsen et al. (1988) over available animal studies. Nielsen et al. (1988) provides concurrent exposure characterization, immunologic findings, and clinically significant outcomes consistent with respiratory sensitization which increase EPA's confidence in its suitability to derive the POD for use in characterizing inhalation risk.

4.3.2 *o*-Phthalic Acid

EPA also evaluated reasonably available information on the human health effects of inhalation exposure to *o*-phthalic acid for use in the general population risk assessment.

EPA identified one *in vivo* study and two *in vitro* assays, all of which were designed to evaluate respiratory sensitization and indicate that *o*-phthalic acid is not a respiratory sensitizer. Sarlo and Clark (1992) demonstrated that *o*-phthalic acid does not induce effective sensitization of the respiratory tract following dermal induction in a study of female Hartley guinea pigs. The study was conducted to evaluate the respiratory sensitization potential of phthalic anhydride, with *o*-phthalic acid used as a negative control as described above in Section 4.3.1. *o*-Phthalic acid has also been evaluated in two *in vitro* assays developed to detect respiratory sensitizers, including the ALIsens assay and GARDair assays described above (NTP, 2026; Invitrolize, 2024). In the ALIsens assay and GARDair assay, *o*-phthalic acid was considered negative for respiratory sensitization, in contrast to phthalic anhydride (Section 4.3.1.1).

Overall, EPA concludes that *o*-phthalic acid is not a respiratory sensitizer. Because there are no studies conducted via the inhalation route for *o*-phthalic acid relevant for extrapolating risk to human health for use in the general population screening assessment (which focuses on risk from exposure to *o*-phthalic acid following releases of phthalic anhydride to the environment), EPA is using the proposed oral POD (HED of 66 mg/kg-day) to extrapolate to the inhalation route as described above in Section 4.1.6. EPA assumes similar absorption for the oral and inhalation routes, and no adjustment was made when extrapolating to the inhalation route. EPA extrapolated the daily oral HEDs to inhalation HECs using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest. Appendix C provides further information on extrapolation of inhalation HECs from oral HEDs. Route-to-route extrapolation of the oral HED to an inhalation HEC results in additional uncertainty. EPA cannot predict whether the assumptions regarding route extrapolation for the chosen POD would lead to over- or underprediction of risk for the dermal and inhalation routes.

5 GENOTOXICITY HAZARD IDENTIFICATION

Limited genotoxicity testing of phthalic anhydride (Table 5-1) and *o*-phthalic acid (Table 5-2) has been conducted. Phthalic anhydride has been evaluated in two bacterial reverse mutation assays, one *in vitro* mouse lymphoma assay, one *in vitro* sister chromatid exchange (SCE) assay, and three *in vitro* chromosomal aberration assays (Table 5-1), but has not been evaluated in any *in vivo* studies. Phthalic anhydride was negative for mutagenicity in both reverse mutation assays with several strains of *Salmonella typhimurium* both with and without metabolic activation (Zeiger et al., 1985). Similarly, phthalic anhydride was negative for SCEs in one study of Chinese hamster ovary (CHO) cells both with and without metabolic activation (Galloway et al., 1987), and was negative for chromosomal aberrations in two studies with CHO cells both with and without metabolic activation (Hilliard et al., 1998; Galloway et al., 1987). In a third chromosomal aberration study with CHO cells, phthalic anhydride induced chromosomal aberrations at the highest dose tested (10 mM) in the absence of metabolic activation; however, the increase in chromosomal aberrations occurred at a dose that resulted in visible precipitate and no increase in chromosomal aberrations was observed in the presence of metabolic activation (Hilliard et al., 1998). Phthalic anhydride was positive for mutagenicity in two trials in an *in vitro* mammalian cell mutagenicity test (mouse lymphoma test) in the absence of metabolic activation at the highest concentration tested (*i.e.,* 200 µg/mL), which coincided with a large inhibition of cellular growth. Negative results reported for phthalic anhydride from *in vitro* assays that conduct tests in aqueous media need to be interpreted with caution given the rapid hydrolysis of phthalic anhydride to *o*-phthalic acid.

o-Phthalic acid has been evaluated in two bacterial reverse mutation assays, two *in vitro* chromosomal aberration assays, one *in vivo* micronucleus study with mice, and one *in vivo* dominant lethal mutation assay with mice (see Table 5-2). *o*-Phthalic acid was negative for mutagenicity in both reverse mutation assays with several strains of *Salmonella typhimurium* both with and without metabolic activation (Lee and Lee, 2007; Zeiger et al., 1992) and did not induce chromosomal aberrations in either in vitro study of CHO cells (Lee and Lee, 2007; Phillips et al., 1982). No increase in micronuclei formation was observed in bone marrow cells from male ICR mice i.p. injected with 0, 20, 100, 500, 2,500, or 12,500 µM/kg o-phthalic acid (Lee and Lee, 2007). In a dominant lethal mutation assay, groups of 20 male mice were i.p. injected with 0, 40, or 80 mg/kg o-phthalic acid for 5 consecutive days and then mated with untreated females for periods of 1 to 7, 8 to 14, 15 to 21 and 22 to 28 days post-treatment (Jha et al., 1998). o-Phthalic acid was found to significantly induce dominant lethal mutations as demonstrated by treatment-related increases in dominant lethality in both dose groups, providing evidence of germ cell mutations. However, results from this assay from this study need to be interpreted with caution and may not be reliable, as it deviated substantially from OECD TG 478 (Rodent Dominant Lethal Test) and these deviations may impact study results (OECD, 2016). As discussed further in Appendix A.2, the primary deviations from OECD TG 478 include: lack of inclusion of a positive control; inadequate number of dose groups (guideline recommends at least 3); inadequate number of animals per dose group to achieve an adequate number of implants (*i.e.,* at least 400); selected doses significantly impacted mating success; and use of an exposure method (*i.e.,* i.p. injection) that is not an intended route of human exposure without specific scientific justification. Furthermore, i.p. injection is generally not considered a relevant route of administration for irritating chemicals, such as *o*-phthalic acid.

Overall, available studies indicate that phthalic anhydride is negative for genotoxicity and mutagenicity in *in vitro* assays, except in one mouse lymphoma assay and one chromosomal aberration assay in the absence of metabolic activation systems at high concentrations that coincided with precipitate formation or high levels of cytotoxicity. Similarly, available studies of *o*-phthalic acid also support the conclusion that *o*-phthalic acid is not genotoxic or mutagenic, except in one dominant lethal mutation assay, which

was considered unreliable due to deviations from OECD TGs that introduced important limitations (OECD, 2016). Similarly, OECD (2005), Australia NICNAS (2013), and Health Canada (2019) have concluded that phthalic anhydride is only genotoxic *in vitro* at high, cytotoxic concentrations, in the absence of a metabolic activation system, and these effects are not expected to be relevant under *in vivo* conditions.

3573 **Table 5-1. Summary of Genotoxicity Studies of Phthalic Anhydride**

Test Type	Test System (Species/ Strain/ Sex)	Dose / Duration	Metabolic Activation	Result	Reference
In vitro – gene mutation studies					
Reverse mutation assay	*S. typhimurium* strains TA 98, 100, 1535, 1537	0, 3.3, 10, 33, 100, 333, 1,000, 3,333 µg/plate phthalic anhydride (provided by Koppers)	± Aroclor 1254 induced rat or hamster liver S9	Negative for mutagenicity	(NTP, 1997; Zeiger et al., 1985) [a]
Reverse mutation assay	*S. typhimurium* strains TA 98, 100, 1535, 1537	0, 1, 3.3, 10, 33, 67, 100, 333, 666 µg/plate phthalic anhydride (provided by Aldrich)	± Aroclor 1254 induced rat or hamster liver S9	Negative for mutagenicity	(NTP, 1997; Zeiger et al., 1985) [a]
Mouse Lymphoma Assay	L5178Y TK ± mouse lymphoma cells	0 (DMSO vehicle), 12.5, 25, 50, 100, 200 µg/mL phthalic anhydride	No activation system used	Positive for mutagenicity (↑ mutant frequency at 200 µg/mL in 2 trials; coincided with large inhibition of cell growth [87–95% inhibition of relative growth compared to vehicle control group])	(NTP, 2021) [a]
In vitro – cytogenetic studies					
SCE Assay	CHO cells	0, 10, 30, 100, 300 µg/mL phthalic anhydride for 2 (+S9) or 25 hours (−S9)	± Aroclor 1254 induced rat liver S9	Negative for SCE	(Galloway et al., 1987) [a]
Chromosomal aberrations	CHO cells	0, 30, 100, 300 µg/mL phthalic anhydride for 2 (+S9) or 8−12 hours (−S9)	± Aroclor 1254 induced rat liver S9	Negative for chromosomal aberrations	(Galloway et al., 1987) [a]
Chromosomal aberrations	CHO cells	Up to 10 mM phthalic anhydride for 3 hours	± Phenobarbital/β-napthoflavone-induced rat liver S9	Negative for chromosomal aberrations (precipitate observed at 8 and 10 mM)	(Hilliard et al., 1998)
Chromosomal aberrations	CHO cells	0, 6, 8, 10 mM phthalic anhydride for 3 hours	± Phenobarbital/β-napthoflavone-induced rat liver S9	Positive for chromosomal aberrations at 10 mM (−S9) (visible precipitate observed) Negative for chromosomal aberrations (+S9)	(Hilliard et al., 1998)

CHO = Chinese hamster ovary; SCE = sister chromatid exchange
[a] Data from NTP study with raw data available through NTP's Chemical Effects in Biological Systems (CEBS) database: https://cebs.niehs.nih.gov/cebs/test_article/85-44-9 (accessed March 26, 2026).

3574 **Table 5-2. Summary of Genotoxicity Studies of *o*-Phthalic Acid**

Test Type	Test System (Species/ Strain/ Sex)	Dose / Duration	Metabolic Activation	Result	Reference
In vitro – gene mutation studies					
Reverse mutation assay	*S. typhimurium* strains TA 97, 98, 100, 1535	0, 33, 100, 333, 1000, 3333, 10,000 µg/plate *o*-phthalic acid	± Aroclor 1254 induced rat or hamster liver S9	Negative for mutagenicity	(Zeiger et al., 1992)
Reverse mutation assay	*S. typhimurium* strains TA 98, 100, 102, 1535, 1537	0, 20, 100, 500, 2500, 12,500 µg/plate *o*-phthalic acid	± Aroclor 1254 induced rat liver S9	Negative for mutagenicity	(Lee and Lee, 2007)
In vitro – cytogenetic studies					
Chromosomal aberrations	CHO cells	0, 20, 100, 500, 2500, 12,500 µM/mL *o*-phthalic acid for 24 hours	± Aroclor 1254 induced rat liver S9	Negative for chromosomal aberrations	(Lee and Lee, 2007)
Chromosomal aberrations	CHO cells	0, 10, 20, 50 mM *o*-phthalic acid for 2 hours	No	Negative for chromosomal aberrations	(Phillips et al., 1982)
In vivo studies					
Micronucleus test	Male ICR mice	Mice (5/dose) i.p. injected with 0, 20, 100, 500, 2500, 12,500 µM/kg *o*-phthalic acid and sacrificed 24 hours later	Not applicable	Negative for micronuclei in bone marrow cells	(Lee and Lee, 2007)
Dominant lethal mutation assay	Male Swiss albino mice	Male mice (20/dose) i.p. injected with 0, 40, 80 mg/kg *o*-phthalic acid for 5 consecutive days and then mated with untreated females for up to 28 days after exposure	Not applicable	Positive for dominant lethal mutations	(Jha et al., 1998)

CHO = Chinese hamster ovary; i.p. = intraperitoneal

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6 CANCER HAZARD IDENTIFICATION

This section discusses available human (Section 6.1) and animal (Section 6.2) evidence for the carcinogenicity of phthalic anhydride. No studies were identified where the test substance was reported as *o*-phthalic acid.

6.1 Summary of Human Evidence

EPA identified one case-control study investigating mortality from lung cancer in workers at the Societa Italiana Serie Acetia Sintetica (SISAS) chemical plant in Italy that produced mainly phthalic anhydride, acetylene, and their derivatives (Riboli et al., 1983). In addition to phthalic anhydride and acetylene, workers were reported to have been exposed to a large number of other chemicals, including several phthalate diesters and soot, which is produced from the partial combustion of methane. Fifty-three male subjects who died between January 1, 1976, and December 31, 1979, and whose death was recorded on the death certificate as due to lung cancer, were chosen as cases. One hundred six referents were chosen from the register as the first two individuals named following each case, provided that their death certificate did not specify respiratory cancer and that their age was within 5 years of the case. Age and death date distributions of the referents were similar to the cases. After controlling for age and smoking habits, authors found an odds ratio for lung cancer mortality of 5.6 (95% confidence limits 1.9–16.2) among workers previously employed at the SISAS facility compared to a group of individuals never occupationally exposed to known lung carcinogens. However, because workers were potentially exposed to a wide range of carcinogens at the facility, the etiology of the cases of lung cancer in former workers could not be conclusively attributed to phthalic anhydride.

6.2 Summary of Animal Evidence

Phthalic anhydride has been evaluated in one 2-year dietary study of F344 rats and one 2-year dietary study of B6C3F1 mice (NCI, 1979). No cancer bioassays are available for the dermal or inhalation exposure routes.

In the first study, male and female F344 rats (20/sex in control; 50/sex in treatment groups) were fed diets containing 0, 7,500, or 15,000 ppm phthalate anhydride (equivalent to approximately 278, 556 mg/kg-day) for 105 weeks (NCI, 1979). No significant increases in pre-neoplastic lesions or tumors were observed in either sex at any dose in any tissue. In the second study, male and female B6C3F1 mice were fed diets containing 0, 25,000, or 50,000 ppm phthalic anhydride based on the results of the dose-range finding study. However, due to excessive body weight loss in both sexes, dietary concentrations of phthalic anhydride were reduced to 12,500 and 25,000 ppm for males and 6,250 and 12,500 ppm for females starting on study week 32 (TWA doses ≈1,817 and 3,634 mg/kg-day for males; 1,336 and 2,672 mg/kg-day for females) (NCI, 1979). No significant increases in pre-neoplastic lesions or tumors were observed in either sex at any dose in any tissue.

As discussed further in Section 4.1.3.1 and Appendix A.1.3, the NCI cancer bioassays of rats and mice had identified issues with the stability of phthalic anhydride in the dosed feed mixtures. NCI (1979) states “Assays of the dosed feed mixtures indicated that they may have been unstable under the conditions of use.” This is based on a stability analysis of feed mixtures containing 15,000 ppm phthalic anhydride that lost 2.59% (or 372 ppm) per day when stored at room temperature. From the analysis, it is unclear if phthalic anhydride was lost due to hydrolysis to *o*-phthalic acid or covalent interactions of phthalic anhydride with proteins in the diet that may have limited test substance availability. NCI (1979) states that feed mixtures were prepared fresh every 1 to 1.5 weeks and diet was routinely stored at 5 °C until its use. Storage of the diet at 5 °C may have slowed phthalic anhydride loss; however, stability was not assessed at 5 °C. Given the identified stability issues, there is uncertainty regarding the precise doses

of phthalic anhydride received by animals in these studies. However, the 2-year NCI studies utilized high doses (375–750 mg/kg-day for rats; 1,803–4,904 mg/kg-day in mice); therefore, even if phthalic anhydride was lost over the 1-to-1.5-week period between preparation of fresh diet, the received doses are still relatively high and EPA considers the study to support qualitative cancer hazard identification. For example, assuming 2.59% loss per day and assuming fresh diet was prepared every 10 days, total loss by the tenth day would be approximately 26%. This means that received doses may have been as low as approximately 278 to 556 mg/kg-day for rats and 1,336 to 2,672 mg/kg-day for mice, assuming 26% loss. Given that the authors provided sufficient information to enable EPA to calculate adjusted received doses, EPA considered this study to be informative as part of the weight of scientific evidence and appropriate for use in quantitative dose-response for non-cancer effects following oral exposures to phthalic anhydride (Section 4.1.6).

6.3 Weight of Scientific Evidence: Conclusions on Carcinogenicity

Under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), EPA reviewed the weight of evidence for the carcinogenicity of phthalic anhydride and preliminarily concluded that phthalic anhydride is *Not Likely to Be Carcinogenic to Humans*. According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a descriptor of *Not Likely to Be Carcinogenic to Humans* is appropriate when:

> …the available data are considered robust for deciding that there is no basis for human hazard concern. In some instances, there can be positive results in experimental animals when there is strong, consistent evidence that each mode of action in experimental animals does not operate in humans. In other cases, there can be convincing evidence in both humans and animals that the agent is not carcinogenic. The judgment may be based on data such as: animal evidence that demonstrates lack of carcinogenic effect in both sexes in well-designed and well-conducted studies in at least two appropriate animal species (in the absence of other animal or human data suggesting a potential for cancer effects), convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans, convincing evidence that carcinogenic effects are not likely by a particular exposure route (see Section 2.3), or convincing evidence that carcinogenic effects are not likely below a defined dose range.

Weight of scientific evidence considerations supporting EPA's draft determination are listed below. Consistent with this cancer classification, EPA is not conducting a dose-response assessment for phthalic anhydride or evaluating phthalic anhydride for carcinogenic risk to humans.

- No evidence of preneoplastic lesions or neoplasms were observed in male or female F344 rats exposed to up to approximately 556 mg/kg-day phthalic anhydride in the diet for 105 weeks (NCI, 1979).
- No evidence of preneoplastic lesions or neoplasms were observed in male or female B6C3F1 mice exposed to up to approximately 3,634 mg/kg-day (males) or 2,672 mg/kg-day (females) phthalic anhydride in the diet for 105 weeks (NCI, 1979).

Similarly, OECD (2005), Health Canada (2019) and Australia NICNAS (2013) have concluded that phthalic anhydride does not cause carcinogenicity in mice or rats, while ACGIH (2025) concluded that phthalic anhydride is "Not Classifiable as a Human Carcinogen" due to the lack of a carcinogenic response in 2-year rodent bioassays.

7 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE

7.1 Hazard Considerations for Aggregate Exposure

Human exposure to phthalic anhydride is expected to occur via the inhalation and dermal routes for workers and consumers (see *Draft Consumer and Indoor Exposure Assessment for Phthalic Anhydride* (U.S. EPA, 2026a) and *Draft Environmental Release and Occupational Exposure Assessment for Phthalic Anhydride* (U.S. EPA, 2026g)). Reasonably available studies provide evidence of sensitization across routes of exposure (*e.g.,* dermal route of entry and respiratory sensitization upon challenge) as further described in Section 4.3.1.1.5. However, the proposed pathway for respiratory sensitization is not as refined and established as that of the OECD dermal sensitization AOP (OECD, 2014); though the AOP for respiratory sensitization (AOP 39) that is under development has many overlaps with that of skin sensitization. EPA has robust confidence that phthalic anhydride is a respiratory sensitizer based on the integration of the available evidence (Section 4.3.1.2), but there are remaining uncertainties regarding the overlap of dermal and respiratory sensitization as separate endpoints. The resulting knowledge gaps reduce the Agency's confidence in aggregating dermal and respiratory exposures to phthalic anhydride. Therefore, EPA does not consider it appropriate to aggregate exposures across routes given the potential differences in toxicity across routes.

For the general population, exposure to *o*-phthalic acid may occur via inhalation, dermal, and oral routes. Because EPA is utilizing screening-level approaches for the general population (see *Draft Environmental Media and General Population and Environmental Exposure for Phthalic Anhydride* (U.S. EPA, 2026f)), aggregating exposures across routes is not appropriate, without first refining exposure scenarios and estimates.

7.2 PESS Based on Greater Susceptibility

EPA addressed subpopulations and lifestages expected to be more susceptible to phthalic anhydride exposure than others. Workers are expected to be more highly exposed to phthalic anhydride than any other population, as discussed in the *Draft Environmental Release and Occupational Exposure Assessment for Phthalic Anhydride* (U.S. EPA, 2026g) and the *Draft Risk Evaluation for Phthalic Anhydride* (U.S. EPA, 2026i). Table 7-1 presents the data sources that were used in the PESS analysis evaluating susceptible subpopulations and identifies whether and how the subpopulation was addressed quantitatively in the draft risk evaluation of phthalic anhydride.

EPA identified plausible differences in susceptibility within exposed worker populations: workers already sensitized to phthalic anhydride are more likely than non-sensitized workers to experience allergic respiratory reactions at lower exposure levels (elicitation) compared with the higher exposures typically required to induce new sensitization (induction). However, due to practical constraints, available epidemiologic studies did not consistently determine workers' sensitization status or its timing (induction versus elicitation), so the evidence for differential susceptibility is indirect. EPA's confidence is supported by complementary immunologic findings (*e.g.,* phthalic anhydride-HSA-specific antibodies) in Nielsen et al. (1991), though these markers do not by themselves distinguish previously sensitized from newly sensitized workers. Collectively, this information is useful as part of the weight of scientific evidence for understanding PESS based on greater susceptibility.

EPA identified indirect evidence for differences among human populations with regard to immune responses. Inter-individual genetic differences may exist that predispose certain individuals toward a particular immune response. For example, atopic individuals (*i.e.,* those with a genetic predisposition to develop allergic reactions via a strong Th2 response) may show greater susceptibility towards

respiratory sensitization (Bae et al., 2011; Fukuyama et al., 2010; Dearman et al., 2000; Dearman and Kimber, 1992; Dearman et al., 1992). Additionally, MHC II genes are highly polymorphic and are thought to influence an individual's susceptibility to allergic disease, including respiratory sensitization (Smit et al., 2014). MHC II molecules are involved in the initiation phase of a sensitization response where DCs present processed antigen to T-cells (*i.e.,* KE 3 in the skin sensitization AOP described in Section 4.2.1.1.3 and KE 3 in the respiratory sensitization AOP described in Section 4.3.1.1.3).

EPA identified evidence of potential differences in susceptibility to dermal sensitization of phthalic anhydride from animal studies (*e.g.,* (Bae et al., 2011; Fukuyama et al., 2010; Dearman et al., 2000; Dearman and Kimber, 1992; Dearman et al., 1992). These studies provide evidence of several factors that enhance susceptibility to dermal exposure to phthalic anhydride, including immune atopy, or genetic predisposition to develop allergy (see also Table 7-1). For example, different strains of mice (*i.e.,* female C57BL/6 mice compared to female BALB/c mice) show different allergic skin reaction responses to dermal application of phthalic anhydride, suggesting a role for genetic factors in differential immune responses. Additionally, modulating the expression of key transcription factors (*e.g., GATA3* and *KFL10*) involved in regulating the balance of T-cell subpopulations alters responses to phthalic anhydride in mice, including increases in allergic skin inflammation responses such as ear thickness, edema, epidermal hyperplasia, number of mast cells. Certain subpopulations may be at elevated risk due to heightened sensitivity to the dermal sensitization hazards of phthalic anhydride.

Table 7-1 summarizes a range of factors that may have the potential to increase biological susceptibility to phthalic anhydride, including genetic polymorphisms, lifestage, pre-existing diseases, physical activity, diet, smoking status, stress, and co-exposures to other environmental stressors that contribute to related health outcomes. Co-exposure to other acid anhydrides (such as TMA) exemplifies the phenomenon of cross-sensitivity, where exposure to chemicals that share functional groups with phthalic anhydride may provoke a sensitization response (IIT Research Institute, 1996). The effect of these factors on susceptibility to health effects of phthalic anhydride is not fully understood. Additionally, although cigarette smoking is a risk factor for sensitization because smoking can increase IgE sensitization to low molecular weight antigens like phthalic anhydride (Tarlo and Lemiere, 2014; Venables et al., 1985), it is unclear if phthalic anhydride elicits respiratory sensitization via an IgE-mediated mechanism and it is unclear how smoking would influence a IgE-independent immune mechanisms. EPA is uncertain about the magnitude of any possible increased risk from effects associated with phthalic anhydride exposure for relevant subpopulations.

For non-cancer inhalation hazards, EPA selected a default factor of 10× to account for intraspecies differences across a population (UF_H) in the draft risk evaluation, including genetic differences that may contribute to different responses in a human population. Consistent with EPA and NRC reports (U.S. EPA, 2014; NRC, 2001), EPA considered two options for refining the toxicokinetic component of the UF_H as described in Section 4.3.1.3.1. As stated in Section 4.3.1.3.1, EPA is soliciting comments from the SACC regarding the underlying assumptions for these options.

For dermal hazards, the uncertainty factor for human variability (UF_H) accounts for increased susceptibility when quantifying risks from exposure to phthalic anhydride. The Risk Assessment Forum, in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), discusses some of the evidence for choosing UFs when data are lacking and describe the types of populations that may be more susceptible, including different life stages (*e.g.,* of children and elderly). EPA applied a UF_H of 1 for dermal hazards because the refined estimate of the POD for phthalic anhydride provided through the use of the SARA-ICE ED_{01} is already considered protective of 99% of the population.

EPA also considers the POD proposed for use in characterizing risks from inhalation exposure to phthalic anhydride to be protective of respiratory sensitization effects in workers. Uncertainty may remain whether additional susceptibility factors would be covered by the default UF_H value of 10 and UF_L of 3 chosen for the draft risk evaluation.

Table 7-1. PESS Evidence Crosswalk for Biological Susceptibility Considerations

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to Phthalic Anhydride		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to Phthalic Anhydride		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Genetics	Target Organ Toxicity	Modulating the expression of key transcription factors (*e.g., GATA3* and *KFL10*) involved in regulating the balance of T-cell subpopulations alters responses to phthalic anhydride in mice, including increases in allergic skin inflammation responses such as ear thickness, edema, epidermal hyperplasia, number of mast cells. Different strains of mice (*i.e.,* female C57BL/6 mice compared to female BALB/c mice) show different allergic skin reaction responses to dermal application of phthalic anhydride, suggesting a role for genetic factors in differential immune responses.	(Bae et al., 2013; Bae et al., 2011; Bae et al., 2010)	Atopic individuals are more likely to become sensitized to phthalic anhydride upon inhalation or dernal exposure. HLA class II polymorphisms contribute to individual susceptibility for occupational asthma induced by low molecular weight agents. Genetic factors that increase the permeability of the stratum corneum.	(Friedmann and Pickard, 2010; Bardana, 2008; Young et al., 1995) (Yuhki and O'Brien, 1990)	Use of default 10× UF_H
Lifestage	Embryos/ fetuses/infants	Developing fetus was less susceptible than dams during pregnancy when pregnant rats were exposed to *o*-phthalic acid from GD 7–16.	(Ema et al., 1997)			Proposed oral POD based on systemic effects (*i.e.,* reduced body weight) is anticipated to be protective.
	Pregnancy/Lactating status	Some evidence of maternal toxicity in pregnant rats exposed to high doses of o-phthalic acid during gestation.	(Ema et al., 1997)			Proposed oral POD based on systemic effects (*i.e.,* reduced body weight) is anticipated to be protective.
	Children	Reduced F1 offspring body weight at 3 months of age was observed in a gestational exposure study (GD 7–16)	(Rahmani et al., 2015)			Proposed oral POD based on systemic effects (*i.e.,* reduced body weight) is anticipated to be protective.

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to Phthalic Anhydride		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to Phthalic Anhydride		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Pre-existing disease or disorder	Health outcome/ target organs	Workers that have previously been sensitized to phthalic anhydride are more susceptible to allergic hypersensitivity reactions compared to newly sensitizer individuals. Workers with preexisting asthma have greater levels of specific IgG against phthalic anhydride-HSA compared to asymptomatic patients; and 1 in 5 workers with asthma had a positive skin prick test against phthalic anhydride-HSA	Nielsen et al. (Nielsen et al., 1991; 1988)	Preexisting conditions may increase susceptibility to adverse respiratory sensitization outcomes (*e.g.,* asthma, viral respiratory tract infections, cystic fibrosis) because a compromised respiratory system could exacerbate the effects of phthalic anhydride. Skin conditions that increase the permeability of the stratum corneum may contribute to adverse dermal sensitization outcomes.	(Bardana, 2008) (Friedmann and Pickard, 2010).	Proposed inhalation POD based on respiratory sensitization is anticipated to be protective because it is based on a study that likely included new and existing cases of sensitization. Qualitative discussion in Section 7.2 and this table
Lifestyle activities	Smoking	No direct evidence identified		Smoking may increase susceptibility for respiratory symptoms such as asthma.	(Zhang et al., 2002) (Tarlo and Lemiere, 2014)	Qualitative discussion in Section 7.2 and this table
	Physical activity	No direct evidence identified		Insufficient activity may increase susceptibility to multiple health outcomes. Overly strenuous activity may also increase susceptibility.	CDC (2022)	Qualitative discussion in Section 7.2 and this table
Sociodemo-graphic status	Socioeconomic status	No direct evidence identified		Individuals with lower incomes may have worse health outcomes due to social needs that are not met, environmental concerns, and barriers to health care access.	ODPHP (2023b)	Qualitative discussion in Section 7.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to Phthalic Anhydride		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to Phthalic Anhydride		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Other chemical and nonchemical stressors	Chemical Co-exposures	Co-exposure to phthalic anhydride and other to other known sensitizers (*i.e.,* TMA, maleic anhydride, isophthalic anhydride), could potentially affect respiratory health (*i.e.,* asthma), hypersensitivity reactions, or lung function. Cross-sensitivity of phthalic anhydride and TMA reported in a study of rats exposed via inhalation to phthalic anhydride and challenged with TMA.	(Nielsen et al., 1991; 1988) (IIT Research Institute, 1996)	Endotoxin exposure may augment response to allergen in individuals already sensitized to a chemical allergen.	(Bardana, 2008)	Qualitative discussion in Section 7.2 and this table
	Built environment	No direct evidence identified		Poor-quality housing may contain environmental triggers of asthma such as pests (*e.g.,* roaches, mice, etc.), mold, dust, or building materials that may exacerbate reduced asthma control associated with phthalic anhydride exposure.	ODPHP (2023a)	Qualitative discussion in Section 7.2 and this table
	Social environment	No direct evidence identified		Social isolation and other social determinants (*e.g.,* decreased social capital, stress) can lead to negative health outcomes.	CDC (2023) ODPHP (2023c)	Qualitative discussion in Section 7.2 and this table

POD = point of departure; UF = uncertainty factor; UF_H = intraspecies UF; HSA = human serum albumin; GD = Gestation Day; F1 = first generation offspring; TMA = trimellitic anhydride

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8 POINTS OF DEPARTURE USED TO ESTIMATE RISKS FROM PHTHALIC ANHYDRIDE EXPOSURE, CONCLUSIONS, AND NEXT STEPS

After considering hazard identification and evidence integration, dose-response evaluation, and weight of scientific evidence of candidate PODs, EPA selected PODs for use in in the draft risk evaluation of phthalic anhydride (U.S. EPA, 2026i). Acute dermal and inhalation PODs for phthalic anhydride were selected for use in the assessments of risk to consumers and workers (Table 8-1). A chronic oral POD was selected for use in the assessment of risk to the general population in the draft risk evaluation of phthalic anhydride (Table 8-2).

EPA considers there to be robust evidence that phthalic anhydride is a skin sensitizer. EPA selected a dermal POD of 45 µg/cm^2 based on skin sensitization. The dermal POD for phthalic anhydride is based on skin sensitization and was estimated using the SARA-ICE model. The SARA-ICE model estimates the ED_{01}, which is the HPPT dermal dose at which there is 1% chance of inducing sensitization (Section 4.2.1.3). The dermal POD will be used to estimate risk from dermal exposure to phthalic anhydride for workers and consumers in the draft risk evaluation of phthalic anhydride (U.S. EPA, 2026i).

As discussed in Section 4.3.1.2, EPA considers there to be robust evidence that phthalic anhydride is a respiratory sensitizer and respiratory sensitization is the most sensitive hazard associated with inhalation exposure to phthalic anhydride. EPA selected an inhalation POD of 0.4 mg/m^3 for phthalic anhydride based on increased respiratory symptoms (*i.e.,* asthma, conjunctivitis, and rhinitis) and increased IgG in exposed worker populations (Nielsen et al., 1988) (Table 8-1). The inhalation POD will be used to estimate risk from inhalation exposure to phthalic anhydride for workers and consumers in the draft risk evaluation of phthalic anhydride (U.S. EPA, 2026i).

For the general population risk assessment, EPA assumed complete and rapid and hydrolysis of phthalic anhydride releases into *o*-phthalic acid occurs in the environment, and therefore derived a chronic oral POD for *o*-phthalic acid. EPA selected an oral POD based on decreased body weight gain in rats as shown in Table 8-2. The oral chronic POD of 278 mg/kg-day (HED = 66 mg/kg-day) for *o*-phthalic acid is based on decreased body weight gain in male F344 rats fed diets containing phthalic anhydride for 2 years (NCI, 1979)(Table 8-2). EPA considers the selected oral chronic POD appropriate for use in its general population risk assessment.

There were no studies of *o*-phthalic acid conducted via the inhalation route relevant for extrapolating human health risk for the general population. In the absence of inhalation studies, EPA performed route-to-route extrapolation to convert the oral HED to an inhalation HEC of 358 mg/m^3 (0.07 ppm). HECs are based on daily continuous (24-hour) exposure, and HEDs are daily values. EPA did not derive a dermal value because dermal exposure to *o*-phthalic acid is not expected to be a relevant exposure to the general population given the physical and chemical properties of *o*-phthalic acid make it unlikely to permeate skin.

EPA is soliciting comments from the SACC and the public on the non-cancer hazard identification, dose-response, and weight of scientific evidence analyses, and the selected hazard values for use in risk characterization of phthalic anhydride. In particular, EPA is seeking SACC and public input on the dose-response assessment used to derive the draft dermal POD based on skin sensitization, including use of the OECD skin sensitization AOP as organizing framework for integration of *in chemico*, *in vitro*, and *in vivo* data, and EPA's application of the SARA-ICE dose-response model. EPA is also seeking input on its use of AOP as an organizational framework for human evidence and evidence from *in chemico*, *in*

vitro, and *in vivo* test systems for respiratory sensitization, as well as its dose-response assessment to derive the draft inhalation POD based on respiratory sensitization.

Table 8-1. Non-Cancer Hazard Values for Phthalic Anhydride for Use in the Human Health Risk Assessment for Workers and Consumers

Relevant Populations in Risk Evaluation	Exposure Scenario	Hazard Value	UFs	Total UFs	Study and Toxicological Effects
Workers ann consumers only	Acute Dermal	HED = 45 µg/cm^2	UF_H = 1	Total UF = 1	Data from 3 LLNAs, 1 DPRAs, and 1 kDPRA were input into the SARA-ICE model to estimate ED01, which is the HPPT dermal dose at which there is 1% chance of inducing sensitization. The POD is the geometric mean of the distribution.
	Acute Inhalation	HEC = 0.4 mg/m^3 = 0.07 ppm	UF_L = 3 UF_H = 10	Total UF = 30	Epidemiological study of factory workers in Sweden. Workers were classified as "heavily" (n = 28 reactor loaders; n = 7 repair men) or "slightly" (n = 25) exposed based on their job. Mean peak air concentrations were 6.6 mg/m^3 in heavily exposed workers, equivalent to an 8-hour TWA of 0.4 mg/m^3 (LOAEL). Mean peak air concentrations in lightly exposed workers were below the limit of detection (0.1 mg/m^3) (Nielsen et al., 1988).

DPRA = direct peptide reactivity assay; HED = human equivalent dose; HEC = human equivalent concentration; HPPT = human predictive patch test; kDPRA= kinetic DPRA; LLNA = local lymph node assay; LOAEL = lowest-observed-adverse-effect level; SARA-ICE = Skin Allergy Risk Assessment – Integrated Chemical Environment; UF = uncertainty factor; UF_A = interspecies UF; UF_H = intraspecies UF; UF_L = LOAEL-to-NOAEL UF

Table 8-2. Non-Cancer Hazard Values for *o*-Phthalic Acid for Use in the Human Health Risk Assessment for the General Population

Relevant Populations in Risk Evaluation	Exposure Scenario	Hazard Value	UFs	Total UFs	Study and Toxicological Effects
General population only	Chronic Oral and Inhalation	HED = 66 mg/kg-day HEC = 358 mg/m^3 = 52.6 ppm	UF_A = 3 UF_H = 10	Total UF = 30	NOAEL of 278 mg/kg-day based on 10% decrease in body weight gain in male F344 rats fed diets containing phthalic anhydride for 2years (NCI, 1979)

HEC = human equivalent concentration; HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; UF = uncertainty factor; UF_A = interspecies UF; UF_H = intraspecies UF

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APPENDICES

Appendix A SUMMARIES OF AVAILABLE EXPERIMENTAL ANIMAL STUDIES OF PHTHALIC ANHYDRIDE

A.1 Repeated Dose Oral Studies

A.1.1 Intermediate (>1–30 Days) Duration Oral Studies

Four studies evaluated health effects following intermediate duration oral exposure to *o*-phthalic acid (all studies conducted with male rats) (Kwack et al., 2010; Kwack et al., 2009; Oishi and Hiraga, 1980; Lake et al., 1975). No intermediate duration oral exposure studies of phthalic anhydride are available.

7-Day Oral (Gavage) Study of Male Wistar Rats (Lake et al., 1975)

Young male Wistar albino rats (6/group) were administered vehicle (corn oil) or 850 mg/kg-day *o*-phthalic acid via gastric intubation for 7 days. The liver was evaluated for biochemical and histological outcomes; effects on other organs were not evaluated. No significant changes on relative liver weight were observed (absolute liver weight and body weights were not reported). Similarly, no significant changes were observed in biochemical parameters in liver homogenates (*i.e.,* succinate dehydrogenase activity, glucose-6-phosphatase activity, aniline 4-hydroxylase activity, biphenyl 4-hydroxylase activity, cytochrome P-450 content, microsomal protein content) following exposure to *o*-phthalic acid. No histopathological or ultrastructural changes in the liver were noted after administration of *o*-phthalic acid (Lake et al., 1975). Overall, this study supports a NOAEL of 850 mg/kg-day with no LOAEL identified, as no effects in the liver were observed in this study at the only dose level evaluated.

7-Day Oral (Dietary) Study of Male Wistar Rats (Oishi and Hiraga, 1980)

Oishi and Hiraga (1980) fed young male Wistar rats control diet (n = 20) or diet containing 2% *o*-phthalic acid for one week (n = 10) (received dose equivalent to ≈2,000 mg/kg-day). Treatment with *o*-phthalic acid did not affect body weight gain or absolute or relative weight of the testes, liver, or kidneys. Testosterone and dihydrotestosterone levels in serum and testosterone levels in testis were not significantly different compared to control animals. Treatment with *o*-phthalic acid did not significantly alter zinc levels in the testes, liver, or serum; however, zinc levels in the kidneys were significantly increased by 11% compared to control animals. No other outcomes were evaluated as part of this study. Overall, this study supports a NOAEL of 2,000 mg/kg-day *o*-phthalic acid with no LOAEL identified.

14-Day Oral (Gavage) Study of Male SD Rats (Kwack et al., 2010)

Male SD rats (n = 6/group) were administered vehicle (corn oil) or 250 mg/kg-day *o*-phthalic acid via gavage for two weeks. All rats were monitored for changes in body and organ weights, clinical chemistry, hematology, and urine parameters. No significant changes were observed in mortality or food consumption. Significant reductions in terminal body weight were observed in male rats (≈14%; estimated from Figure 2 in Kwack et al.). No significant changes were observed in relative weight of any organs evaluated (*i.e.,* adrenal gland, heart, kidney, lung, liver, spleen, thymus, thyroid, testis, or epididymis). Absolute organ weight was not reported. No significant effects were observed on any hematological parameters (*i.e.,* red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count) or clinical chemistry parameters (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, creatine phosphokinase, lactate dehydrogenase, total protein, albumin, glucose, total cholesterol, total bilirubin, triglyceride, blood urea

nitrogen, creatinine, calcium, potassium, sodium, chloride). No urinalysis parameters (*i.e.,* red blood cells, bilirubin, urobilinogen, ketone, protein, nitrite, glucose, pH, specific gravity, leukocytes) were significantly altered following treatment with *o*-phthalic acid. Overall, this study supports a LOAEL of 250 mg/kg-day *o*-phthalic acid for a 14% reduction in body weight with no NOAEL identified; however, the reduction in body weight was not accompanied by any other signs of toxicity (Kwack et al., 2010).

28 Day Oral (Gavage) Study of Male SD Rats (Kwack et al., 2009)

Four-week-old male SD rats (6/group) were administered vehicle (corn oil) or 250 mg/kg-day *o*-phthalic acid via oral gavage for four weeks. All rats were monitored for changes in body and organ weights, clinical chemistry, hematology, sperm analysis and urine parameters. No mortality or significant differences in food consumption were observed in the *o*-phthalic acid treatment group. Body weight was significantly reduced in male rats starting after 6 days of *o*-phthalic acid administration and remained reduced for the remainder of the study. Mean terminal body weight was reduced by approximately 22% (estimated from Figure 2 in Kwack et al.). Treatment with *o*-phthalic acid did not significantly affect the relative weight of any organs (*i.e.,* adrenal gland, heart, kidney, lung, liver, spleen, thymus, thyroid, testis, or epididymis). Absolute organ weight was not reported. Compared to controls, treatment with *o*-phthalic acid did not significantly affect any hematological (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count) or clinical chemistry parameters (total protein, albumin, glucose, total cholesterol, total bilirubin, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, alkaline phosphatase, blood urea nitrogen, triglyceride, γ-glutamyl transferase). The right cauda epididymis from each animal was collected and sperm counts and motility were assessed. Compared to controls, *o*-phthalic acid did not significantly affect the mean sperm count, mean percent motile sperm, or other sperm motility parameters (*i.e.,* average path velocity, straight-line velocity, amplitude of the lateral head displacement, beat cross frequency, straightness, linearity). The only motility parameter altered by *o*-phthalic acid treatment was curvilinear velocity, which was reduced (33%) from 261.28 µm/s in control sperm to 174.05 µm/s in sperm from animals treated with *o*-phthalic acid. Overall, this study supports a LOAEL of 250 mg/kg-day *o*-phthalic acid for reduced (22%) body weight; however, the reduction in body weight was not accompanied by any other signs of toxicity (Kwack et al., 2009).

A.1.2 Subchronic (>30–90 Days) Duration Oral Exposure Studies

Three subchronic duration oral exposure studies were identified, including one of *o*-phthalic acid with male rats (Murakami et al., 1986) and two studies of phthalic anhydride (1 each of rats and mice) (NCI, 1979).

34 to 36 Day Oral (Dietary) Study of Male Wistar Rats (Murakami et al., 1986)

Male Wistar rats were fed powder diet containing 0, 0.5, or 5% *o*-phthalic acid for 34 to 36 days (precise duration of study for control and *o*-phthalic acid treated animals not provided). Equivalent received doses were estimated to be approximately 500 and 5,000 mg/kg-day *o*-phthalic acid. There was no effect on body weight gain throughout the study, terminal body weight, or absolute and relative organ weight (*i.e.,* liver, kidney, spleen, and testes) following dietary exposure to *o*-phthalic acid. There was also no effects on the activity of several liver enzymes in isolated liver mitochondria, including that of succinate, pyruvate, or glutamate dehydrogenase. Similarly, no changes were observed in other serum biochemical parameters following exposure to *o*-phthalic acid, including alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), cholesterol, α-globulin, β-globulin, $_{\gamma}$-globulin, lactate dehydrogenase, albumin-to-globulin ratio, albumin, triglycerides, uric acid, and creatine phosphokinase (CPK). No histopathologic changes in the liver, kidney, or testes

of rats treated with *o*-phthalic acid. Overall, this study supports a NOAEL of 5,000 mg/kg-day *o*-phthalic acid with no LOAEL identified.

7 Week Oral (Dietary) Study of F344 Rats (NCI, 1979)

A 7-week feeding study with F344 rats was conducted to estimate the maximum tolerated dose of phthalic anhydride for a subsequent 2-year cancer bioassay (summarized below in Appendix A.1.3). Male and female F344 rats (5/sex/dose) were fed diets containing 0, 6,200, 12,500, 25,000, or 50,000 ppm phthalic anhydride for 7 weeks, followed by a 1-week observation period (equivalent received doses ≈230, 463, 926, 1,853 mg/kg-day). Animal body weight was recorded twice weekly and unspecified tissues were examined microscopically. All animals survived untiled scheduled necropsy. Terminal bodyweight was reduced in high-dose male and female rats by 24 to 26% at study week 7 (male and female body weights across dose groups: 90, 95, 92, 74% control for males; 95, 93, 91, 76% control for females). Histopathologic findings were limited to the livers of male rats in the 25,000-ppm group (trace amounts of centrilobular cytoplasmic vacuolation was observed in 4 out of 5 males); however, tissues were normal in high-dose (50,000 ppm) male and female rats. No further study details were provided. Overall, this study supports a NOAEL of 926 mg/kg-day and a LOAEL of 1,853 mg/kg-day based on reduced (24−26%) body weight in male and female rats. Based on the findings of this study, NCI (1979) set the low- and high-dose groups at 7,500 and 15,000 ppm for the subsequent 2-year cancer bioassay of F344 rats (summarized below in Appendix A.1.3).

7 Week Oral (Dietary) Study of B6C3F1 Mice (NCI, 1979)

A 7-week feeding study with B6C3F1 mice was conducted to estimate the maximum tolerated dose of phthalic anhydride for subsequent 2-year cancer bioassays (summarized below in Appendix A.1.3). Male and female B6C3F1 (5/sex/dose) were fed diets containing 0, 6,200, 12,500, 25,000, or 50,000 ppm phthalic anhydride for 7 weeks, followed by a 1-week observation period (equivalent received doses were ≈692, 1,389, 2,779, or 5,558 mg/kg-day). Animal body weight was recorded twice weekly and unspecified tissues were examined microscopically. All animals survived untiled scheduled necropsy. Terminal body weight was unaffected by treatment with phthalic anhydride. No histopathologic findings were reported in male or female mice at up to 50,000 ppm. No further details were provided. Overall, this study supports a NOAEL of 5,558 mg/kg-day with no LOAEL identified. Based on findings of this study, NCI (1979) set the low- and high-dose groups at 25,000 and 50,000 ppm for the subsequent 2-year cancer bioassay of B6C3F1 mice (summarized below in Appendix A.1.3).

A.1.3 Chronic (>90 Days) Duration Oral Exposure Studies

Two chronic carcinogenicity studies were identified that also evaluated cancer and non-cancer health effects following exposure to phthalic anhydride (Kluwe, 1986; NCI, 1979).

2 Year Oral (Dietary) Study of F344 Rats (Kluwe, 1986; NCI, 1979)

Male and female F344 rats (20/sex in control group; 50/sex in treatment groups) were fed a diet containing 0, 7,500 or 15,000 ppm phthalic anhydride (≈278 and 556 mg/kg-day) for 105 weeks. Animals were examined daily for deaths and clinical signs of toxicity. Macroscopic and microscopic examinations were conducted for all major tissues and organs (*e.g.,* skin, lungs, spleen, liver, kidney, heart, brain, urinary bladder, testis, prostate, mammary gland, thyroid, uterus, ovary, etc.). Organ weight, serum biochemistry, and hematology were not evaluated as part of this study. Survival to the end of the study was comparable across dose groups for males (survival: 70, 88, 72%) and females (survival: 85, 84, 82%). Low incidence of clinical observations (*i.e.,* arched back, rough hair coat, ulceration, corneal opacity) were reported in male and female rats dosed with phthalic anhydride; however, incidence data were not provided. Body weight gain was unaffected in female rats of both dose

groups, and in low-dose males. In high-dose males, body weight was lower than that of the control group from study week 13 to the end of the study. These data are presented as growth curves (see Figure 1 of (NCI, 1979)), where the reduction in body weight gain appeared slight at most timepoints (≈10%). However, the exact percent reduction in body weight for high-dose males was not reported.

Histologic examinations revealed no significant neoplastic or non-neoplastic findings that could be attributed to phthalic anhydride in male or female rats at either dose level. Under the conditions of the bioassay, NCI concluded that "there was no conclusive evidence for the carcinogenicity of phthalic anhydride in F344 rats…" Overall, this study supports a NOAEL of 278 mg/kg-day based on a slight reduction (approximate 10%) in body weight in male rates at the LOAEL of 556 mg/kg-day.

2 Year Oral (Dietary) Study of B6C3F1 Mice (Kluwe, 1986; NCI, 1979)

Male and female B6C3F1 mice (20/sex in control group; 50/sex in treatment groups) were fed diets containing phthalic anhydride for 104 weeks. For the first 32 weeks of the study, dietary concentrations of phthalic anhydride were 25,000 and 50,000 ppm. However, due to excessive body weight loss, dietary concentrations of phthalic anhydride were reduced to 12,500 and 25,000 ppm for males and 6,250 and 12,500 ppm for females for the remainder of the study. TWA dietary concentrations were 16,346 and 32,692 ppm (estimated to be ≈1,817 and 3,634 mg/kg-day based on a food factor of 0.15) for males and 12,019 and 24,038 ppm (≈1,336 and 2,672 mg/kg-day based on a food factor of 0.15 and adjusted based on author reported loss (26%) of phthalic anhydride in feed) for females. Macroscopic and microscopic examinations were conducted for all major tissues and organs (*e.g.,* skin, lungs, spleen, liver, kidney, heart, brain, urinary bladder, testis, prostate, mammary gland, thyroid, uterus, ovary, etc.). Organ weight, serum biochemistry, and hematology were not evaluated as part of this study. Survival was not significantly affected by chronic exposure to phthalic anhydride in males (survival: 85, 74, 94%) or females (survival: 80, 90, 80%). Dose-related decreases in body weight gain were observed in low- and high-dose mice of both sexes with terminal body weight reduced by 12 to 27% in low and high-dose mice of both sexes. No treatment-related neoplastic changes were observed in any tissue or treatment group. NCI (1979) concluded that there were no statistically significant treatment-related non-neoplastic pathological effects in either male or female mice. However, the U.S. EPA (1988) IRIS program re-evaluated the histopathology data and found significant increases in the incidence of several non-neoplastic lesions in the lung, kidney, bile duct, adrenal gland, and brain/thalamus of low- and high-dose male and female mice (Table_Apx A-1). Overall, this study supports a LOAEL of 1,817 mg/kg-day for male mice and 1,336 mg/kg-day for female mice based on histologic findings, reduced body weight gain, and reduced terminal body weight, with no NOAEL identified.

Although 2-year studies by NCI (1979) support a NOAEL of 278 mg/kg-day, based on reduced male rat body weight gain and a LOAEL (no NOAEL identified) of 1,336 mg/kg-day for female mice based on histologic findings and reduced body weight, these studies cited potential issues with the stability of phthalic anhydride in the dosed feed mixtures. NCI (1979) states, "Assays of the dosed feed mixtures indicated that they may have been unstable under the conditions of use." This is based on a stability analysis of feed mixtures containing 15,000 ppm phthalic anhydride that lost 2.59% (or 372 ppm) per day when stored at room temperature. From the analysis, it is unclear if phthalic anhydride was lost due to hydrolysis to *o*-phthalic acid or covalent interactions of phthalic anhydride with proteins in the feed that may have limited test substance availability. Loss is not expected to be due to volatilization, as phthalic anhydride is non-volatile (U.S. EPA, 2026h). Furthermore, NCI (1979) states that feed mixtures were prepared fresh every one-to-one and half weeks and diet was routinely stored at 5 °C until its use. Storage of the diet at 5 °C may have slowed phthalic anhydride loss, however, stability was not assessed at 5 °C. Given the identified stability issues, there is uncertainty regarding the doses of phthalic anhydride received by animals is these studies. However, consideration of the author-reported

information that allowed EPA to calculate adjusted doses accounting for loss of test substance (assume 25.9% loss) is described in Section 4.1.3, which reflects the adjusted doses.

Table_Apx A-1. Incidence of Histopathologic Lesions in B6C3F1 Mice Fed Diets Containing Phthalic Anhydride for 2 Years (NCI, 1979) [a]

Organ	Histopathologic Finding	Control	Low-Dose	High-Dose
Male mice				
Lung	Lymphocytosis	6/20[b] (30%)	19/50 (38%)	30/49 (61%)
Kidney	Lymphocytosis	0/20	15/50 (30%)	37/49 (76%)
Bile Duct	Chronic Inflammation	1/20 (5%)	7/50 (14%)	17/49 (35%)
Adrenal Cortex	Atrophy	0/20	23/49 (47%)	40/48 (83%)
Brain/Thalamus	Mineralization	0/19	18/50 (36%)	23/49 (47%)
Female mice				
Lung	Lymphocytosis	2/20 (10%)	32/49 (65%)	34/48 (71%)
Kidney	Lymphocytosis	0/20	22/48 (46%)	26/48 (54%)
Bile Duct	Chronic Inflammation	10/20 (50%)	30/48 (63%)	36/48 (75%)

[a] Incidence data from Tables D1 and D2 of NCI (1979).
[b] Indicates the number of animals with the lesion over the number of animals examined histologically.

A.2 Studies of Developmental and Reproduction

The developmental toxicity of phthalic anhydride has been evaluated in one study of mice (Fabro et al., 1982), while the developmental toxicity of *o*-phthalic acid has been evaluated in two studies of rats (Rahmani et al., 2015; Ema et al., 1997). No one or two generation studies of reproduction are available for phthalic anhydride or *o*-phthalic acid.

The Relative Teratogenic Index and Teratogenic Potency: Proposed Components of Developmental Toxicity Risk Assessment (Fabro et al., 1982)

In a non-guidelines study, Fabro et al. (1982) treated pregnant CD-1 mice with daily intraperitoneal (i.p.) injections of phthalic anhydride in 0.5% carboxymethyl cellulose solution on GD8 through GD10. The precise number of treatment groups and precise exposure levels were not documented by study authors; however, authors do state that "The highest dose level for teratology studies was normally within the 95% confidence limits of the calculated LD01 concentration, and a geometric progression of doses below this level was administered until no significant effects were observed…For the majority of compounds, four or more dose levels were utilized, and in all cases there were at least 10 dams per group." Dams were sacrificed on day 18 of pregnancy, and all fetuses were examined for viability and number, resorptions, and gross malformations. Adult lethality was investigated in an independent experiment in which non-pregnant mice were administered i.p. injections of phthalic anhydride on 3 consecutive days and then monitored for lethality for up to 2 weeks after the final injection. Adult LD01 and LD50 values were reported to be 0.37 mmol/kg-day (95% CI: 0.19−0.43 mmol/kg-day) and 0.51 mmol/kg-day (95% CI: 0.44−0.57 mmol/kg-day), respectively. The tD_{05} and tD_{50} values, defined as the "doses require to induce an additional 5 or 50% malformation rate above the background" were extrapolated to be 0.40 (95% CI could not be calculated) and 1.37 mmol/kg-day (95% CI could not be calculated). Teratogenic effects were observed at doses that also caused lethality in non-pregnant female mice (*i.e.,* LD_{01} and tD_{05} overlap). The relevance of these findings is questionable due to poor documentation of administered doses and dose groups, the fact that teratogenicity was observed at doses that also caused maternal lethality, and the human relevance of the route of exposure.

Prenatal Oral (Dietary) Study of Pregnant Wistar Rats (Ema et al., 1997)

Pregnant Wistar rats (Wistar-Kyoto) were exposed to *o*-phthalic acid (0, 1.25, 2.5, or 5.0%) on GD 7 through GD 16 (11 dams/dose group) via diet. Dams were observed daily for clinical signs of toxicity, and maternal body weight and food consumption were recorded daily. The average daily intake of *o*-phthalic acid was calculated to be 1,021, 1,763, and 2,981 mg/kg-day for the 1.25, 2.5, and 5.0% groups, respectively. Pregnant rats were sacrificed on day 20 of pregnancy. No mortality or clinical signs of toxicity were observed in any treatment group. Maternal weight gain was reduced by 18 to 59% in a dose-dependent manner in the 2.5% and 5.0% treatment groups compared to control animals on GD 7 through GD 16. Adjusted maternal body weight gain (*i.e.,* maternal weight gain excluding the gravid uterus) was also reduced in a dose-dependent manner but only reached statistical significance in the high dose group, with mean adjusted weight gain values of 50 g (control), 47 g (1.25%), 42 g (2.5%), and 30 g (5.0%). Mean maternal food consumption was reduced by 13% (2.5%) to 27% (5.0%) compared to control animals on GD 7 through GD 16.

Treatment with *o*-phthalic acid did not induce any significant changes at any dose level in the number of litters, number of corpora lutea or implantations per litter, number of litters totally resorbed, number of resorptions and dead fetuses per litter, incidence of post-implantation loss per litter, the number of live fetuses per litter, or the sex ratio of live fetuses. Mean body weight of live male fetuses was significantly reduced by approximately 4% in the high-dose group (5%) compared to the control, while the body weight of live female fetuses was not significantly affected at any dose level. External and internal examinations revealed no malformations in any treatment group. Skeletal examinations revealed a slight reduction in the number of ossification centers of caudal vertebrae in fetuses from the high-dose group (5%) (mean # of ossification centers = 5.1) compared to control fetuses (mean # of ossification centers = 5.5). Overall, this study supports a maternal NOAEL of 1,021 mg/kg-day based on reduced maternal body weight gain and food consumption and a developmental NOAEL of 1,763 mg/kg-day based on a slight (4%) decrease in body weight of male fetuses and a slight (8%) reduction in the number of ossification centers of caudal vertebrae. Although generally well-conducted, this study has several limitations. First, it included fewer dams per dose group than recommended by current OECD TG No. 414 (11 vs. 20 dams per dose group). Second, the exposure window did not cover late gestation up to parturition, as recommended by current OECD TG No. 414 (OECD, 2018b).

Prenatal Oral (Dietary) Study of Wistar Rats (Rahmani et al., 2015)

Rahmani et al. (2015) evaluated the structure and function of the heart in 3-month-old pups following prenatal exposure to *o*-phthalic acid. Pregnant Wistar rats (8/group) were fed diets containing 0, 2.5, and 5.0% *o*-phthalic acid (equivalent to ≈0, 1,763, and 2,981 mg/kg-day) on GD 7 through GD 16. No maternal (*e.g.,* body weight gain, food consumption) or reproductive (*e.g.,* litter size, numbers of resorptions or post-implantation loss, sex ratio, etc.) parameters were reported. For the majority of measured pup parameters (unless otherwise stated), study authors do not state whether or not the observed effects are for male, female, or combined male and female pups. Pup bodyweight was reduced by 14.5% and 25.4% in the 1,763, and 2,981 mg/kg-day groups, respectively. Absolute heart weight was reduced in both dose groups; however, when normalized to bodyweight, relative heart weight increased in the high dose group (5%). Mean systolic blood pressure (≥2.5%) and heart rate (5%) increased in a dose-dependent manner. Thoracic aorta wall thickness increased when unnormalized and normalized to inner wall diameter (≥2.5%) and the cross-sectional area of the thoracic aorta increased (≥2.5%) in a dose-dependent manner. Similarly, the wall thickness (unnormalized and normalized to the inner diameter) and cross-sectional area of the septal branch of the left descending coronary artery were increased in a dose-dependent manner, while the inner diameter of the artery deceased (≥2.5%). In male offspring, levels of malondialdehyde increased (≥2.5%), while superoxide dismutase activity,

glutathione peroxidase activity, nitric oxide synthetase activity decreased (≥2.5% *o*-phthalic acid) in heart tissue in a dose-dependent manner.

Similar to Ema et al. (1997), there are several limitations associated with the study of Rahmani et al. (2015), including fewer dams per dose group than recommended by current OECD TG No. 414 (*i.e.,* 8 vs. 20 dams per dose group); the exposure window did not cover late gestation up to parturition, as recommended by current OECD TG No. 414; and information that could be used to determine maternal toxicity was not reported, including dam body weight, clinical signs, and food consumption throughout gestation. This reporting deficiency could impact the interpretation of the results.

Dominant Lethal and Sperm Abnormality Assays of o-Phthalic Acid in Mice (Jha et al., 1998)

Jha et al. provide data from three separate experiments conducted in male Swiss albino mice: one acute duration lethality study and two intermediate duration mutagenicity studies of dominant lethality and sperm abnormality). Jha et al. first conducted a lethality study to determine the maximum tolerated dose (MTD) following i.p. injection. Mice (15/group) were given single doses of 0 (vehicle control;10% DMSO in PBS), 100, 200, 300, 400, or 500 mg/kg *o*-phthalic acid, and then monitored for survival and clinical signs for 30 days. At 500 mg/kg, 12 out of 15 mice died within 3 days of the injection. At 400 mg/kg, 8 out of 15 mice died within 22 days of the injection. All mice in the 0, 100, 200, and 300 mg/kg groups survived the 30-day observation period. The authors based the MTD of 400 mg/kg on transient clinical signs of toxicity (*e.g.,* "lack of appetite, tardiness, etc.") in the 300 and 400 mg/kg groups, which were initially observed and gradually disappeared (no further information was provided).

For the dominant lethal mutation assay, mice (20/group) were given i.p. injections of 0 (10% DMSO in PBS), 40, or 80 mg/kg *o*-phthalic acid for 5 consecutive days (total doses = 0, 200, and 400 mg/kg *o*-phthalic acid). After treatment, each male was caged with two untreated female mice, which were replaced weekly for four consecutive weeks. Females were checked for vaginal plugs daily to determine pregnancy. Uterine contents were inspected for live and dead implants during pregnancy days 14 to 16. General signs of toxicity during the study (*i.e.,* clinical signs, food consumption, body weight gain) was not reported for males. Decreases in the percent of pregnant females were observed during all four weekly mating intervals in the *o*-phthalic acid dose groups (% pregnant females across dose groups [Week 1: 90, 87.5, 80%; Week 2: 90, 82.5, 75%*; Week 3: 90, 75*, 65%*; Week 4: 85, 80, 55%*], * = significantly different from control at $p < 0.05$), indicative of impaired reproductive performance. Other changes that were observed following *o*-phthalic acid treatment included decreased number of total implants, decreased number of implants per female, decreased live implants per female, and increased dead implants per female in both treatment groups; changes were statistically significant throughout mating intervals two, three, and four. The percent dominant lethality increased dose-dependently throughout all four mating intervals, leading study authors to conclude that *o*-phthalic acid is a germ cell mutagen.

Limitations exist that impact the ability to interpret the results of the dominant lethal mutation assay. The study did not meet acceptability criteria as outlined in paragraph 37 of OECD TG No. 478 (Rodent Dominant Lethal Test) (OECD, 2016). First, the study did not include an appropriate positive control, which under OECD TGs should always be included, unless the laboratory has recently demonstrated proficiency in the assay, which study authors do not report. Second, the study did not include an adequate number of dose groups (*i.e.,* at least 3 in addition to control) to assess dose-response or number of animals to obtain an adequate number of total implants (*i.e.,* at least 400 total implants per dose group per mating interval). Third, criteria for dose selection were inconsistent with OECD guidelines. For example, the MTD is defined at the highest dose that is tolerated without evidence of study-limiting toxicity that would necessitate humane euthanasia and should not adversely affect mating success;

however, study authors did not evaluate animals for signs of toxicity or clinical signs in the dominant lethal mutation study. Furthermore, mating success in both dose groups was decreased (*i.e.,* decreased percent of pregnant females during each mating trial). Finally, study authors do not scientifically justify use of i.p. injection, which the OECD TGs state, "is not normally recommended since it is not an intended route of human exposure, and should only be used with specific scientific justification." Further, *o*-phthalic acid is also an irritant, therefore observed effects, such as reduced mating success, may be attributed to local irritation at the site of injection (*i.e.,* the peritoneal cavity).

The third experiment evaluated sperm abnormality in mice (n = 5/group) given a single i.p. injection of 0 (10% DMSO in PBS), 100, 150, 200, or 300 mg/kg *o*-phthalic acid. Smears of spermatozoa from epididymides were evaluated for abnormal sperm 1, 3, and 5 weeks after exposure. A statistically significant increase in the incidence of sperm head abnormalities was observed at doses of 100 mg/kg-day and up at 1 and 3 weeks (spermatozoa and spermatid stages). The authors report that the most common types were, "amorphous, elongate, without hook, and giant amorphous." At 5 weeks, a significant increase in abnormal sperm was only observed in the high-dose group (300 mg/kg-day).

A.3 Sensitization and Irritation

Quantitative DPRA and kDPRA of Phthalic Anhydride (Wareing et al., 2017)

Wareing et al. evaluated the skin sensitization hazard of phthalic anhydride in the quantitative DPRA and DPRA. The quantitative DPRA was performed in accordance with OECD TG 442C (OECD, 2023a), with the exception that in addition to the standard test concentration of 100 mM, phthalic anhydride was also tested at 1- and 10-mM concentrations. The reaction time was 24 hours and the estimated phthalic anhydride concentration required to cause 6.38% cysteine and lysine peptide depletion (EC6.38 cysteine/lysine prediction model) was estimated. Across the tested concentrations (1, 10, 100 mM) cysteine depletion was 1.46, −2.93, and −3.94% , lysine depletion was 1.5, 10.83, 32.06%, and mean peptide depletion was 1.48, 5.41, 16.03%. The EC6.38 value for cysteine and lysine depletion was 18.22 mM phthalic anhydride.

For the kDPRA, concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 mM phthalic anhydride was evaluated in the presence of 0.5 mM of cysteine peptide (final ratios of peptide: phthalic anhydride = 1:10, 1:5, 2:5, 4:5, 8:5). Peptide depletion was then evaluated after 5, 10, 30, 60, 120, and 240 minutes. Reaction rate constants (k values) were then calculated for each incubation time, with k values of 0.2122, 0.0906, 0.0349, 0.0120, 0.0049, and 0.0015 s^{-1} M^{-1} after 5, 10, 30, 60, 120, and 240 minutes, respectively. The logarithm of the maximum k value (*i.e.,* $k = 0.2122$ s^{-1} M^{-1}; $\log k = -0.67$) was compared to a threshold value of $\log k = -1.73$ to discriminate between GHS CLP Cat 1A and 1B. Using this approach, phthalic anhydride was classified ap GHS CLP Cat 1A for skin sensitization.

DPRA, U937-CD86 Assay (U-SENS), and KeratinoSens Assay of Phthalic Anhydride (Natsch et al., 2013)

Natsch et al. evaluated the skin sensitization hazard of phthalic anhydride using the DPRA, U937-CD86 Assay, and KeratinoSens Assay. The DPRA was conducted generally in accordance with OECD TG 442C (OECD, 2023a). Briefly, lysine and cysteine depletion was evaluated by high-performance liquid chromatography after a 24-hour reaction time. The final reaction contained 0.5 mM of the peptide and 5 or 25 mM of phthalic anhydride, which equates to molar ratios of 1:10 and 1:50. Phthalic anhydride caused 1.9 and 75% depletion of cysteine and lysine, respectively, with an overall DPRA depletion of 38.45%. Under the conditions of the assay, phthalic anhydride was classified as a sensitizer.

The U937-CD86 assay (also referred to as the U937 Cell Line Activation Test or U-SENS) was generally conducted in a manner consistent with OECD TG 442E (OECD, 2023b). Briefly, 100 µL of U937 cells (human myelomonocytic cells) in a suspension of 5×10^5 cells per mL were plated into 96-well flat bottom plates. 100 µL of phthalic anhydride was then added to the cell suspension. Study authors report that 4 to 6 concentrations of each test substance were tested based on solubility and resulting cell viability, however, study authors do not report the exact concentrations of phthalic anhydride that were tested. Cells were then incubated at 37 °C for 48 hours, before being harvested and stained with fluorescein isothiocyanate (FITC)-conjugated anti-human CD86 monoclonal antibody and FITC-isotype control IgG1. Cells were then assessed for viability (propidium iodide staining) and CD86 expression via flow cytometry. A SI was calculated as the isotype corrected percent CD86+ test divided by the isotype corrected percent CD86+ control multiplied by 100. SI values greater than 150 were considered a positive test result. Under the conditions of the test phthalic anhydride was considered to give a positive result and the concentration of phthalic anhydride that induces a SI of 150 (EC150 value) was estimated by linear regression to be 1,080 µM phthalic anhydride (or 159.95 µg/mL based on a molecular weight of 148.1 g/mol).

The KeratinoSens Assay generally conducted consistent with OECD TG 442D (OECD, 2024). Briefly, KeratinoSens transgenic cells were grown for 24 hours in 96-well plates and then medium was replaced with medium containing phthalic anhydride and a final concentration of 1% DMSO. Phthalic anhydride was tested at 12 concentrations ranging from 0.98 to 2,000 µM in four replicate plates, including 1 that was prepared for determining cytotoxicity. Cells were incubated for 48 hours and then luciferase activity and cytotoxicity (with MTT-assay) were determined. $EC_{1.5}$ and EC_3 values were determined (*i.e.,* values representing the concentration for which induction of luciferase activity is 1.5 and 3-fold), as was the IC_{50} value (*i.e.,* concentration for with a 50% reduction of cellular viability occurs). $EC_{1.5}$, EC_3, and IC_{50} values for phthalic anhydride were all greater than 2,000 µM (highest dose tested).

DPRA, LuSens, KeratinoSens, Modified Myeloid U937 Skin Sensitization Test (mMUSST), and h-CLAT of Phthalic Anhydride (Bauch et al., 2012)

Bauch et al. evaluated phthalic anhydride in DPRA, LuSens, KeratinoSens, mMUSST, and h-CLAT assays. The DPRA assay was conducted in a manner consistent with OECD TG 442C (OECD, 2023a). Briefly, a test concentration of 100 mM phthalic anhydride was evaluated for cysteine and lysine depletion following a 24-hour reaction time (peptide-to-phthalic anhydride ratio 1:10 for cysteine and 1:50 for lysine). A threshold value of 22.62% cysteine and lysine depletion was set in accordance with OECD TG 442C to discriminate between low and moderation reactivity. Phthalic anhydride caused 16.7 and 31.3% depletion of cysteine and lysine, respectively, with an overall DPRA depletion of 24.0%. Under the conditions of the assay, phthalic anhydride was classified as having moderation peptide reactivity.

The ARE/Nrf2 based LuSens and KeratinoSens reporter gene assays can be used to indirectly assess the intracellular cysteine reactivity of chemicals and the subsequent activation of the Keap-1/Nrf2 signaling pathways. Phthalic anhydride was evaluated in both assays in a manner generally consistent with OECD TG 442D (OECD, 2024). In both assays the response was below the threshold for activation, and phthalic anhydride was considered to give a negative response.

The mMUSST (also referred to as the U937 Cell Line Activation Test or U-SENS) and h-CLAT assays can be used to assess DC activation. The mMUSST measures the cell surface protein CD86, while the h-CLAT measures the cell surface proteins CD86 and CD54 as measures of maturation of DCs. Both assays were generally conducted in a manner consistent with OECD TG 442E (OECD, 2023b). In both

assays the response was below the threshold for activation, and phthalic anhydride was considered to give a negative response in both assays.

h-CLAT of Phthalic Anhydride (Nukada et al., 2012)

Nukada et al. evaluated phthalic anhydride in the h-CLAT assay in a manner consistent with OECD TG 442E (OECD, 2023b). Briefly, THP-1 cells were cultured in 24-well plates with eight different concentrations of phthalic anhydride for 24 hours (max concentration tested: 400 µg/mL). After the 24-hour treatment, cells were washed and incubated with FITC-conjugated monoclonal antibodies for anti-human CD54, anti-human CD86, and FITC labeled-mouse IgG1. Cells were then analyzed for protein expression of CD54 and CD86 by flow cytometry. The maximum relative fluorescence intensity (RFI) for CD86 and CD54 were 115 and 160, respectively. No EC150 for CD86 expression or EC200 for CD54 expression could be calculated. Under the conditions of the assay, phthalic anhydride was considered to give a negative response.

Myeloid U937 Skin Sensitization Test (U-SENS) of Phthalic Anhydride (Piroird et al., 2015)

Piroird et al. evaluated phthalic anhydride and 174 other chemicals in the *in vitro* U-SENS assay in a manner consistent with OECD TG 442E (OECD, 2023b). Only results for phthalic anhydride are further discussed. Briefly, human myeloid U937 cells (cell line CRL-159.2) were cultured in 96-well plates with 0, 1, 10, 20, 50, 100, and 200 µg/mL phthalic anhydride for 45 hours. Positive (picrylsulphonic acid at 50 µg/mL) and negative (lactic acid at 200 µg/mL) controls were included in each experiment and the experiment was completed two times. After the 45-hour exposure, cytotoxicity and CD86 expression were determined with incubation of cells with anti-CD86 antibody and subsequent flow cytometry analysis. Overall, phthalic anhydride was predicted to be a non-sensitizer with an EC150 value (*i.e.,* the estimated concentration showing a 150% increase of CD86 expression) for CD86 expression of greater than 200 µg/mL and a CV70 value (*i.e.,* the estimated concentration showing 70% cell viability) of greater than 200 µg/mL. Study authors speculated that the false-negative result may have been the result of the rapid hydrolysis of phthalic anhydride to the non-sensitizer *o*-phthalic acid in the aqueous cell culture medium.

LLNA of Phthalic Anhydride (Dearman et al., 2000)

Dearman et al. evaluated phthalic anhydride in a LLNA and evaluated cytokine production of cells from draining lymph nodes in mice sensitized with phthalic anhydride. For the LLNA, BALB/c mice (4/dose) were exposed topically on the dorsal side of both ears to 25 µL of 0, 0.1, 0.25, 0.5, 1, and 2.5% phthalic anhydride for 3 consecutive days. Five days post-initiation of topical application of phthalic anhydride, mice were injected intravenously via the tail vein with 20 µCi of [^{3}H]TdR in 0.25 mL PBS. Five hours later the auricular lymph nodes were excised and lymphocyte proliferation was evaluated via β-scintillation counting. Treatment with phthalic anhydride causes a dose-dependent increase in the SI (SI: 1, 1.98, 4.75, 4.81, 6.01, 10.78 across dose groups) and an EC3 value of 0.16% phthalic anhydride was estimated.

To investigate cytokine production, BALB/c mice (10 for control; 5 for treatment group) were administered 50 µL of a 1 molar solution of phthalic anhydride bilaterally on each shaved flank. Five days later the treatment was repeated. Five days later 25 µL of phthalic anhydride or vehicle was applied to the dorsal side of both ears daily for three consecutive days. Thirteen days after the initiation of the exposure to phthalic anhydride, draining auricular lymph nodes were excised and single-cell suspensions of lymph node cells were prepared. Levels of interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-12 (IL-12), and interferon-$_\gamma$ (IFN- $_\gamma$) were then evaluated in supernatants after 72, 96, and 120 hours. Treatment with phthalic anhydride resulted in treatment-related increases in IL-10 and IL-12 (but not IL-

4 or IFN-$_{\gamma}$) in supernatant at all evaluated timepoints. Study authors concluded that phthalic anhydride could elicit a Th2-type cytokine secretion phenotype.

LLNA of Phthalic Anhydride (van Och et al., 2000)

van Och et al. evaluated phthalic anhydride for dermal sensitization in an LLNA. Six- to eight-week-old BALB/c mice (3/group, pooled sexes) were pretreated with 1% sodium dodecyl sulphate (SDS) (w/v) for one hour, and then exposed to 25 µL of vehicle (4:1 acetone/olive oil) or 0.25, 1, 2.5, 10, or 25% phthalic anhydride on both ears for 3 consecutive days. Three days post-final topical application of phthalic anhydride, the auricular lymph nodes were removed and lymphocyte proliferation was evaluated in pooled lymph nodes from each animal. Briefly, single cell suspensions were prepared by pressing the lymph node through a 70 µm nylon cell strainer, and then cultured at a concentration of 1×10^7 cells/mL in round-bottomed 96-well plates with 10 µL of [^{3}H]TdR for 24 hours. [^{3}H]TdR incorporation was then determined by liquid scintillation counting in a β plate counter.

Treatment with 1% SDS did not elicit a positive response compared to vehicle only treated animals. Lymphocyte proliferation was observed in response to exposure to all concentrations of phthalic anhydride, and an EC3 value (estimated concentration that elicits a SI of 3) of 0.357% (95% CI: 0.226−0.560) was calculated.

LLNA of Phthalic Anhydride (Plitnick et al., 2003)

Female BALB/c mice (8−12 weeks old; 5/dose) were administered 12.5 µL of vehicle (acetone:olive oil) or phthalic anhydride at concentrations of 0.15, 1.5, and 15% on both sides of each ear (25 µL total per ear) for 3 consecutive days. Mice were then given 1 rest day followed by an intravenous injection of 20 µCi ^{3}H-TdR via the tail vein. Five hours after the injection, mice were sacrificed and auricular lymph nodes were harvested and pooled. Single cell suspensions were then generated and ^{3}H-TdR incorporated was evaluated to estimate the SI. The SI was above the threshold of 3 in mice treated with 1.5 (SI ≈ 10) and 15% (SI ≈ 25) phthalic anhydride indicating a positive response, but not those treated with 0.15% phthalic anhydride. Study authors did not estimate an EC3 value.

In another study, female BALB/c (8–12 weeks old; 5–6/dose) were administered 100 µL of 2.5% phthalic anhydride on the shaved flanks on study days 0 and 5. On study days 10, 11, and 12, mice were then administered 12.5 µL of 2.5% phthalic anhydride to each side of both ears. Two days later (study day 14), mice were sacrificed and auricular lymph nodes were excised and total RNA was exacted from these tissues for determination of Th2 cytokine mRNA. Expression of mRNA for IL-2, IL-3, IL-4, IL-5, IL-10, IL-13, IL-15, and IFN-$_{\gamma}$ were unaffected by treatment with phthalic anhydride. Study authors repeated the experiment with a higher concentration of phthalic anhydride (15%). In the second experiment, the higher concentration of phthalic anhydride induced increased expression of IL-4, IL-10, and IL-13 mRNA.

LLNA and Guinea-Pig Maximization Test (GPMT) of Phthalic Anhydride (Basketter and Scholes, 1992)

Basketter et al. evaluated phthalic anhydride for skin sensitization potential using the GPMT and the LLNA. For the GPMT, Albino Dunkin Hartly guinea pigs were injected with a series of 6 intradermal injections of 0.1% phthalic anhydride in the shoulder region to induce sensitization. Six to eight days post induction injections, an occluded patch containing 25% phthalic anhydride (vehicle = acetone-polyethylene glycol [70:30, v/v]) was placed at the injection site for 48 hours. Twelve to fourteen days after the induction patch was removed, animals were challenged by placing an occluded patch containing 10% phthalic anhydride on one flank for 24 hours. Skin was scored for erythema and oedema 24 and 48 hours after removal of the challenge patch. Ninety percent of guinea pigs gave a positive

response for skin sensitization at 24 and/or 48 hours and phthalic anhydride was classified as an "extreme" sensitizer.

In the LLNA, Basketter et al. treated groups of CBA/Ca mice with vehicle (acetone-olive oil (4:1 v/v), 2.5, 5, or 10% phthalic anhydride via a daily topical application of 25 µL of each test concentration on the dorsal surface of each ear for 3 consecutive days. Four to five days after the first topical application of phthalic anhydride, mice were injected intravenously through the tail vein with phosphate buffered saline containing [^{3}H]methyl thymidine and then sacrificed five hours later and lymphocyte proliferation was assessed. The ratio of test substance to control lymphocyte proliferation ranged from 20.9 to 26, indicating that phthalic anhydride is a sensitizer. No EC3 value (estimated concentration that elicits a SI value of 3) could be calculated.

Buehler Test (3- and 9-Induction Procedures) of Phthalic Anhydride (Botham et al., 2005)

Botham et al. conducted standard and modified Buehler tests to evaluate phthalic anhydride for dermal sensitization. One- to three-month-old male and female guinea pigs (Hartley Crl:HA; 10/sex/group) were clipped and/or shaved at the application site 18 to 24 hours prior to application of the vehicle (acetone) or phthalic anhydride. During the induction phase, vehicle or 20% phthalic anhydride was applied cutaneously to the shaven anterior left flank once per week (standard Buehler method) or 3 times per week (modified Buehler method) for three consecutive weeks. Filter paper loaded with test substance was applied to the shaven flank and held in contact with skin for 6 hours by an occlusive dressing. For the modified Buehler test, the concentration of phthalic anhydride was reduced from 20 to 10% starting with the fifth application due to the severity of observed cutaneous reactions. Following the induction period, animals received no treatment for 11 days. For the challenge phase, animals treated with phthalic anhydride were cutaneously exposed to 20% phthalic anhydride (a 20% challenge was used for all groups) on the shaven posterior right flank via occlusive dressing for 6 hours, while vehicle was applied to the shaven posterior left flank via occlusive dressing for 6 hours. Cutaneous reactions were then evaluated 24, 48, and 72 hours after the challenge. In the control group, a persistent discrete or moderate erythema (grade 1 or 2) was reported in 2 out of 10 animals, which the study authors speculated may be attributable to the irritating properties of phthalic anhydride, while the standard and modified Buehler tests produced 85 and 65% positive reaction rates indicating that phthalic anhydride is a sensitizer. Considerations and limitations of the standard and modified Buehler tests include that these topical application methods can be less sensitive compared to those that use injection of test material and adjuvant. Additionally, the study by Botham cannot differentiate whether phthalic anhydride elicits a type I (*i.e.,* IgE-mediated) or type IV (*i.e.,* T-cell mediated) hypersensitivity response.

Immune Responses of Cynomolgus Monkeys to Phthalic Anhydride (Biagnini et al., 1988)

Biagini et al. evaluated the sensitization potential of phthalic anhydride using young male cynomolgus monkeys. Monkeys were split into four treatment groups (4/group), all of which received 10 consecutive weekly subcutaneous injections of 2 mg aluminum hydroxide, plus weekly injections of one of the following: 200 µg of phthalic anhydride conjugated to monkey serum albumin (phthalic anhydride-MSA, group 1); 200 µg of phthalic anhydride (in ethanol-saline vehicle, group 2); 200 µg of MSA (group 3); and (4) ethanol-saline alone (group 4). Subcutaneous injections were administered for 10 consecutive weeks. At biweekly intervals over the 10-week experiment, serum specific IgG to phthalic anhydride-MSA and specific IgE to phthalic anhydride-MSA were determined via the enzyme-linked immunosorbent assay (ELISA) and radioallergosorbent test (RAST) methods, respectively. Direct intracutaneous skin testing was also performed biweekly via intracutaneous injection of Evans blue dye and observance of cutaneous bluing reactions. Total serum IgE and phthalic anhydride-specific IgE were not significantly different between treatment groups at any time point. However, IgE has a relatively low serum concentration and shorter half-life, which could make it more difficult to detect. Additionally,

there is individual variability in immune responses to antigens, where individuals can preferentially mount an IgG response versus an IgE response. Phthalic anhydride-specific IgG levels were significantly elevated in monkeys administered phthalic anhydride-MSA (group 1) after 4, 6, 8, and 10 weeks of treatment, compared to all other groups. Monkeys treated with conjugated phthalic anhydride-MSA (group 1) had significantly greater phthalic anhydride-MSA skin test results after 8 and 10 weeks of treatment compared with the other treatment groups. Based on these results, study authors concluded that phthalic anhydride MSA injections can induce positive skin sensitivity.

Divergent Immune Responses to Respiratory and Contact Chemical Allergens: Antibody Elicited by Phthalic Anhydride and Oxazolone (Dearman and Kimber, 1992)

Adult (8−12 weeks old) female BALB/c mice (5−10/group) were administered 50 µL of 50, 10, 5, or 1% phthalic anhydride dissolved in 4:1 acetone:olive oil (vehicle) bilaterally on the shaved flanks. Seven days later 25 µL of 50, 10, 5, or 1% phthalic anhydride was applied to the dorsal side of both ears. Enzyme-linked immunosorbent assays (ELISA) were then used to evaluate IgE and presence of IgG anti-hapten antibody in serum. Eight, 14, and 21 days following initiation of treatment, serum IgG anti-hapten antibody was found to be significantly increased in the 50, 10, and 5% treatment groups, but not the 1% treatment group. For experiments evaluating serum IgE, mice were exposed to a single dose level of phthalic anhydride (25%) using the same exposure paradigm described above. Treatment with 25% phthalic anhydride caused a significant increase in serum IgE 8, 14, and 21 days after the initiation of treatment, with the maximal response observed on day 14. Further analysis of isotype distribution of IgG anti-hapten antibody was conducted for mice treated with 25% phthalic anhydride. At the 8-, 12-, and 21-day timepoints, phthalic anhydride induced a significant IgG1 and IgG2b antibody responses, while the IgG2a antibody response was much across all timepoints compared to the IgG2b response (ratio of IgG2a:IgG2b ranged from 1:2.71 to 1:4.18).

Variable Effects of Chemical Allergens on Serum IgE Concentration in Mice; Preliminary Evaluation of a Novel Approach to the Identification of Respiratory Sensitizers (Dearman et al., 1992)

Young female BALB/c mice (4/group) were exposed topically on the dorsum of both ears to 25 µL of 25% phthalic anhydride or vehicle alone. Three days later, mice were injected intravenously via the tail vein with [^{3}H]methyl thymidine, and then 5 hours later mice were sacrificed and the draining auricular lymph nodes were excised and pooled for each group. SI values were then determined based on [^{3}H]methyl thymidine incorporation. Under the conditions of this assay, the SI was approximately 10.

In a second experiment, groups of 10 female mice were administered 50 µL of 25% phthalic anhydride on each shaved flank. Seven days later, 25 µL of 25% phthalic anhydride diluted 1:1 with vehicle was applied to the dorsum of both ears. Mice were then sacrificed 8-, 14-, or 21-days post-exposure and serum IgE concentrations were determined. Serum levels of IgE were significantly increased compared to naïve control animals at all evaluated timepoints.

Effect of Th2 Cytokine Antagonist Treatments on Chemical-Induced Allergic Response in Mice (Ban and Hettich, 2005)

Female Balb/c mice (n = 12/group) were dermally exposed to phthalic anhydride (12.5% and 6.25% for sensitization and challenge, respectively) or vehicle (4:1 acetone-olive oil, v/v). For the sensitization phase, phthalic anhydride was applied to both shaved flanks on the first 4 days of the study. For the challenge phase, phthalic anhydride was applied to the dorsum of both ears after day 7. Four days later (day 11) serum levels of IgE and IgG2a were determined. Significant increases were observed in the serum levels of IgE on study day 7 following sensitization with phthalic anhydride (12.5%), as well as levels of IL4, IL10 in *ex vivo* splenocyte cultures. Serum levels of IgE were also increased on day 11

after challenge with phthalic anhydride (6.25%). No significant changes were observed in *ex vivo* splenocyte production of IL-2, IFN-γ, nor serum levels of IgG2a. There were limitations regarding exposure characterization, including the unclear nature of when the challenge occurred; authors provide enough information to ascertain that it was after 7 but before day 11 of the study. Additionally, the study only examined one dose of phthalic anhydride (12.5%) for the sensitization phase via intradermal injection, which limits its utility for dose-response evaluation.

Age-Related Response Of IL-4/Luc/Cns-1 Transgenic Mice to Phthalic Anhydride Exposure (Sung et al., 2016)

In a mechanistic study by Sung et al. (2016) the effect of aging on allergic inflammation that results from phthalic anhydride exposure was examined. Young (2-month-old) and old (12-month-old) IL-4/Luc/CNS-1 transgenic (Tg) mice (n = 5-6/group) were dermally exposed to vehicle (100 µL of acetone-olive oil) or phthalic anhydride (100 µL 15% solution) on the dorsum of the ear and shaved back skin 3 times per week for 2 weeks. The IL-4/Luc/CNS-1 Tg mice have a reporter construct that induces the production of luciferase when IL-4 is expressed. Evaluated outcomes included measurements of ear thickness and ear morphology, organ weight, serum IgE levels, histopathology of the ear, and protein expression of cytokines, including IL-6 and VEGF. Additionally, luciferase expression was quantified in the mesenteric lymph node (ML), thymus and pancreas as a readout of the production of IL-4. However, the authors do not report data for control animals for this latter endpoint, which impacts the ability to interpret this result.

Decreased absolute thymus weight was observed in young mice (57%) and old mice (50%) exposed to phthalic anhydride compared to vehicle controls. Increases in absolute mesenteric lymph node weight (63%) and spleen weight (81%) were observed in young mice exposed to phthalic anhydride, but not in old mice. In young and old mice, increased ear thickness were observed following exposure to phthalic anhydride, but there was no significant difference between old and young phthalic anhydride groups. The ears of mice that had been dermally exposed to phthalic anhydride appeared to have a darker tint and thicker ear vein compared to vehicle controls, but there were no changes in ear morphology were noted between young and old mice. Increased thickness of the epidermis and dermis (determined via histopathological analysis of H&E staining of ear tissue) were observed for both young and old mice exposed to phthalic anhydride. These findings coincided with increased concentrations of serum IgE as well as number of mast cells, and IL-6 and VEGF expression in ear homogenates from young and old mice exposed to phthalic anhydride. Of note, there were significant differences in the cytokine expression levels between young and old mice exposed to phthalic anhydride.

Following dermal exposure to phthalic anhydride, luciferase signals were detected in the mesenteric lymph node, thymus, and pancreas, indicating expression of IL-4. Luciferase expression was higher in the mesenteric lymph node and pancreas of old mice exposed to phthalic anhydride compared to the young mice exposed to phthalic anhydride. A major limitation and reporting deficiency is that the authors do not report the results for vehicle controls for this endpoint, precluding the ability to interpret the effect of phthalic anhydride on IL-4 from these data. Other limitations include a small sample size, failure to report the sex of the animals, and the inclusion of only one dose in the study design.

LLNA for Phthalic Anhydride in Conjunction with RT-PCR and ELISA for Interferon-g and Interleukin-4 (Vandebriel et al., 2000)

Male and female BALB/c mice (6–8 weeks old; n = 4 to 8/group) were sensitized with 25 µL phthalic anhydride (25%) or vehicle (4:1 acetone:olive oil) for 3 consecutive days via dermal application to the dorsum of both ears. Five days later, the auricular lymph nodes (*i.e.,* local lymph nodes) were excised and evaluated for weight, cell number, lymphocyte proliferation, and levels of cytokines (IFN-γ and IL-

4; gene expression via qPCR and protein content via ELISA). Increased weight of the local lymph nodes corresponding with increased lymphocyte proliferation was observed following exposure to phthalic anhydride. In parallel, increased levels of IL-4 and IFN-γ were observed. The authors evaluated these outcomes in other sensitizers, including the known contact allergen dinitrochlorobenzene (DNCB) and the respiratory allergen trimellitic anhydride (TMA). Limitations of the study included the inclusion of only a single dose of phthalic anhydride, relatively low sample size, and qualitative reporting of the increase in lymph node weights following phthalic anhydride exposure (*i.e.,* quantitative data not shown).

GATA Binding Protein 3 Overexpression and Suppression Significantly Contribute to the Regulation of Allergic Skin Inflammation (Bae et al., 2011)

Bae et al. (2011), evaluated the involvement of GATA3 in phthalic anhydride-induced allergic skin inflammation. The GATA binding protein 3 (Gata3) is a zinc finger transcription factor that regulates the balance in the ratio of Th1 helper T-cells to Th2 helper T-cells, which is thought to be indicative of the pathogenesis of allergic diseases such as asthma and atopic dermatitis. GATA3 is required for the differentiation of CD4+ T-cells into the Th2 lineage, and promotes the production of Th2 cytokines including IL-4, IL-5, IL-6, and IL-13. Wild-type and transgenic mice overexpressing human *GATA3* (Tg mice) were exposed to vehicle (4:1 v/v acetone-olive oil) or phthalic anhydride (50 µL of 1, 5 or 10% solution) on the dorsum of the ear 3 times a week for 3 weeks. The authors provided data on ear thickness as a measure of inflammation, histopathology of the ear and skin, absolute weights of the draining auricular lymph nodes, and serum levels of Igs and cytokines for wild-type and Tg mice exposed to increasing concentrations of phthalic anhydride or vehicle.

Increased ear thickness, epidermal thickness, and inflammatory cell number were observed in wild-type mice exposed to 5% phthalic anhydride compared to wild-type mice exposed to vehicle alone. Increased ear thickness was also observed for high-dose (*i.e.,* 10%) wild-type mice compared to wild-type vehicle controls; the other endpoints did not report results for the high-exposure group (*i.e.,* 10% group). Increases in ear and skin thickness corresponded with a visible dose-dependent increase in absolute weights of the auricular lymph nodes, as well as Th2 Ig concentrations (*e.g.,* IgE, IgG1), and secretion of Th2 type cytokines (*e.g.,* IL-4, IL-6). Secretion of Th1 type cytokines (*e.g.,* INF-γ) was suppressed under the same conditions. Statistical analyses were not reported for these comparisons of exposed wild-type mice versus vehicle control wild-type mice, which is a limitation of the study. The effects of phthalic anhydride in wild-type mice were augmented in Tg mice, demonstrating the involvement of GATA3 in allergic skin inflammation of phthalic anhydride.

IL-6, VEGF, KC and RANTES Are a Major Cause of a High Irritant Dermatitis to Phthalic Anhydride in C57BL/6 Inbred Mice (Bae et al., 2010)

In Bae et al. (2010), female C57BL/6 and BALB/c mice were dermally exposed to vehicle (40 µL of acetone-olive oil) or phthalic anhydride (40 µL of 5% solution) on the dorsum of the ear and shaved back skin 3 times per week for 4 weeks. The authors provided data on ear thickness, absolute weights of the auricular lymph nodes, serum Ig levels, and cytokines from homogenized ear tissue. Histopathological data of the back skin were also provided (*i.e.,* back skin score), represented as a categorical quantification of the degree of irritant dermatitis, considering erythema, hemorrhage, edema, excoriation, erosion and dryness. The authors chose BALB/c and C57BL/6 mice for the study because they are IgE high responders and IgE low responders, respectively.

In BALB/c and C57/BL6 mice, the authors reported increases in ear thickness and skin score following exposure to phthalic anhydride, indicative of allergic skin inflammation. These responses were higher in C57BL/6 mice, which suggests this strain may be more sensitive to phthalic anhydride than BALB/c

mice. Increases in absolute weight of the auricular lymph nodes and concomitant increases in serum IgE levels were observed in both BALB/c and C57/BL6 mice, but responses were higher in BALB/c mice. Increases in cytokine levels (*i.e.,* IL-6, VEGF, KC and RANTES) were observed in BALB/c mice and C57BL/6 mice exposed to phthalic anhydride.

Phthalic Anhydride-Induced Skin Inflammation Is Augmented in KLF10-Deficient Mice Respiratory Sensitization (Bae et al., 2013)

In a letter to the editor, Bae et al. (2013) reported additional evidence of allergic skin inflammation following dermal exposure to phthalic anhydride. Mice with a genetic deficiency of *Kruppel-like factor 10 (KLF-10*) were generated from backcrossing C57BL/6 with129/SV mice. Female WT and KFL10 (- /-) mice (6/group) were dermally exposed to vehicle (40 µL of acetone-olive oil) or phthalic anhydride (40 µL of 1% or 5% solution) on the dorsum of the ear and shaved back skin 3 times/week for 3 weeks. Data were provided for ear thickness, back skin score, absolute weights of the auricular lymph nodes, and protein expression for IL-6, pp38, pERK, and PKC-delta in homogenized ear tissue. Of note, the authors do not provide evidence that confirms their genetic knockdown was achieved (*e.g.,* q-PCR data, gene or protein expression data of KLF-10), which substantially limits any interpretation of these results, especially those regarding a mechanistic involvement of *KLF-10*.

Nevertheless, the authors provide data which demonstrates increased ear thickness and increased skin thickness, increased infiltration of mast cells, and increased absolute weight of the auricular lymph node following dermal exposure to phthalic anhydride (5%) in WT mice. In parallel, increased absolute lymph node weight was observed in WT mice exposed to phthalic anhydride (both 1 and 5%). Increased IL-6, PKC, and PERK protein levels were observed in ear tissue homogenates from WT mice exposed to phthalic anhydride.

There were several additional limitations of this study, including lack of reporting for all dose levels for all prespecified outcomes (*e.g.,* epidermal thickness and infiltration of mast cells is only reported for 5% *o*-phthalic acid, not 1%), which limits the ability to understand the dose-response across all endpoints of the report.

A Tier Approach for Evaluating the Respiratory Allergenicity of Low Molecular Weight Chemicals (Sarlo and Clark, 1992)

Sarlo et al. evaluated phthalic anhydride and/or *o*-phthalic acid for respiratory allergenicity in three studies. In the first study, female Hartley guinea pigs (10/group) were injected subcutaneously two times per week for 4 weeks with 400 µL of 6.7×10^{-3} M, 6.7×10^{-4} M, or 6.7×10^{-5} M phthalic anhydride; 6.7×10^{-4} M *o*-phthalic acid; or vehicle (*i.e.,* olive oil). After a 1-week rest period, animals were injected with an additional 400 µL of phthalic anhydride, *o*-phthalic acid, or vehicle, and then animals were given an additional 1-week rest period. On first day following the second rest period, serum was collected, respiratory reactivity was evaluated on the second day, and skin testing was performed on day four. Immediate-onset respiratory reactivity was evaluated by intratracheally administering 100 µL of challenge antigen (500 µg/mL of conjugate) and then monitoring animals for changes in breathing patterns over a ten-minute period. Diaphragmatic contractions occurring at a minimum of every 36 to 40 normal breaths over the 10-minute observation period was considered an indication of a significant respiratory reaction. No respiratory reactions were observed in control animals, whereas 60 to 90% of animals in the phthalic anhydride treatment groups had significant respiratory reactions following intratracheal challenge.

In a second study, Sarlo et al. evaluated *o*-phthalic acid for respiratory allergenicity. Female Hartley guinea pigs (10/group) were injected subcutaneously two times per week for 4 weeks with 400 µL of

6.7×10^{-4} M *o*-phthalic acid or vehicle (*i.e.,* olive oil). After a one-week rest period, animals were injected with an additional 400 µL of *o*-phthalic acid or vehicle, and then animals were given an additional one-week rest period. On the first day following the second rest period, serum was collected, respiratory reactivity was evaluated on the second day, and skin testing was performed on day four. Immediate-onset respiratory reactivity was evaluated by intratracheally administering 100 µL of challenge antigen (500 µg/mL of conjugate), and then monitoring animals for changes in breathing patterns over a 10-minute period. Diaphragmatic contractions occurring at a minimum of every 36 to 40 normal breaths over the 10-minute observation period was considered an indication of a significant respiratory reaction. No respiratory reactions were observed in control or *o*-phthalic acid treated animals following intratracheal challenge.

In a third study, Sarlo et al. exposed guinea pigs (5–6/group) to 0.05 to 0.2 and 0.6 to 6 mg/m^3 phthalic anhydride dust for three hours per day for 5 consecutive days. Phthalic anhydride dust concentrations were reported as a range due to the day-to-day difficulty in controlling the dust levels in the exposure chambers. During the experiment, animals were housed individually in whole body plethysmographs. Two weeks after the final exposure, control and phthalic anhydride dust exposed animals were challenged with aerosolized phthalic anhydride-GPSA (guinea pig serum albumin) conjugate for 30 minutes and respiratory measurements were made. A positive respiratory reaction was defined as an increase in the respiratory rate and/or peak breath height that were greater than three standard deviations from the mean change in control animals, and the response must be sustained for at least five minutes to be considered positive. *No antibody responses or immediate-onset respiratory reactions were observed in animals exposed to 0.05 to 0.2 mg/m^3 phthalic anhydride dust (NOAEC), whereas positive respiratory reactions were observed for all six animals exposed to 0.6 to 6 mg/m^3 phthalic anhydride dust (LOAEC).* Additionally, animals exposed to 0.6 to 6 mg/m^3 phthalic anhydride dust produced IgG and allergic antibodies (IgG1a) to *o*-phthalic acid-GPSA compared to control animals.

Induction of Type I Hypersensitivity in Guinea Pigs After Inhalation of Phthalic Anhydride (Sarlo et al., 1994)

Sarlo et al. (1994) provide data to suggest that exposure of guinea pigs to *o*-phthalic acid dust at levels as low as 0.5 mg/m^3 can sensitize animals to produce an allergic response. Female Hartley smooth-haired guinea pigs (8–16/group) were exposed via whole-body inhalation to 0.5, 1, or 5 mg/m^3 phthalic anhydride dust in 1 m^3 stainless steel chambers for 3 hours per day for 5 days. Measured chamber concentrations were 0.55, 1.27, or 5.57 mg/m^3 ; MMAD = 3.12 ± 2.02 µm, 3.26 ± 1.96 µm, 3.91 ± 2.08 µm. Two weeks after the last exposure, guinea pigs were challenged with phthalic anhydride dust (control and 5.0 mg/m^3; n = 8/group) or phthalic anhydride-GPSA conjugate (control, 0.5, 1, and 5.0 mg/m^3; n = 8) and respiratory rate was monitored using a plethysmograph. The animals challenged with phthalic anhydride dust were exposed for 30 minutes in a head out plethysmograph (TWA = 5.53 mg/m^3). The animals challenged with phthalic anhydride-GPSA conjugate were exposed to 2 mg/m^3 aerosolized phthalic anhydride-GPSA conjugate in a whole-body plethysmograph for 30 minutes. No challenge with GPSA aerosol was performed due to undefined technical difficulties, which is a limitation of the study. Respiratory rate was monitored 30 minutes prior to the challenge, during the 30-minute challenge, and up to 60 minutes after the challenge. A significant immediate-onset respiratory reaction was defined as an increase in respiratory rate greater than three standard deviations from the mean change of air control animals. Serum levels of IgG were determined via ELISA, and circulating allergic antibodies (IgG1a and IgE) were determined via the dye-based method of passive cutaneous anaphylaxis testing. Gross pathology and histopathology of the lungs of all animals exposed and challenged with 5 mg/m^3 phthalic anhydride dust were examined 24 hours after *o*-phthalic acid challenge.

No significant changes in respiratory rate were observed in animals from the 5 mg/m^3 phthalic anhydride dust compared to air controls following challenge with aerosolized phthalic anhydride-GPSA conjugate. Significant and sustained increases in respiratory rate were observed in the 0.5 and 1 mg/m^3 groups (one animal in each group) as well as the 5 mg/m^3 group (4 animals) compared to the air control groups following challenge with the 2 mg/m^3 aerosolized phthalic anhydride-GPSA conjugate. Similarly, significant respiratory reactions were observed in the 0.5 mg/m^3 group (1 animal), and 5 mg/m^3 group (3 animals) that resulted in significant increases in plethysmograph pressure in these animals. These respiratory reactions coincided with dose-dependent increases in serum levels of IgG antibody across all phthalic anhydride dust groups, reaching significance at all exposure levels.

Levels of IgE were undetectable in all animals. Levels of IgG1a antibody specific to phthalic anhydride-GPSA (anti-phthalic anhydride IgG1a) was elevated in animals challenged with 2 mg/m^3 aerosolized phthalic anhydride-GPSA conjugate (*i.e.,* 0.5 mg/m^3 group [3/8 animals]; 1.0 mg/m^3 [1/8]; 5 mg/m^3 [5/8]). In animals challenged with 5 mg/m^3 phthalic anhydride dust, one animal had detectable IgG1a antibody.

Histopathological findings in the lungs of animals challenged with 5 mg/m^3 phthalic anhydride included hemorrhagic lung foci. An average of 115 lung foci were observed, albeit with large variability (mean ± SE: 115 ± 157), compared to 1 in air controls (Mean ± SE: 1 ± 1). Microscopic examination revealed alveolar hemorrhage with accumulation of red blood cells and some minimal type II cell hyperplasia. No lung foci were observed in animals challenged with *o*-phthalic acid-GPSA aerosol and no microscopic examination was conducted for these animals. Although this study reports quantitative lung histopathology, the large variance in the dataset is a limitation that increases uncertainty. Additional limitations and considerations of this study include the lack of proper controls (*i.e.,* GPSA-only exposure controls). Overall, this study supports a LOAEC of 5 mg/m^3 based on histopathology of the lung, or a NOAEC/LOAEC of 1.0/5.0 mg/m^3 based on increased change in respiratory rate and plethysmograph pressure. The LOAEC is further supported by increased levels of IgG1a and the dose-dependent increase in IgG antibody in animals exposed to and challenged with 5 mg/m^3 phthalic anhydride dust.

The Respiratory LLNA as a Tool to Study Respiratory Sensitizers (Arts et al., 2008) and Contact and Respiratory Sensitizers Can Be Identified by Cytokine Profiles Following Inhalation Exposure (De Jong et al., 2009)

Two studies by the same research group compared the sensitization potential and potency of phthalic anhydride across inhalation and dermal routes. The authors evaluated sensitization following inhalation exposure and following dermal exposure to phthalic anhydride, and published results from the same animals across each study (De Jong et al., 2009; Arts et al., 2008).

For the inhalation exposure, male BALB/c mice (5–12/group) were exposed nose-only to 15 mg/m^3 phthalic anhydride (measured concentration: 14.2 ± 1.5 mg/m^3; MMAD = 2.6 ± 4.4 µm) or vehicle (acetone) for 360 minutes per day for 3 consecutive days. The authors referred to this as a respiratory LLNA. A dermal LLNA was also conducted, in which male BALB/c mice (3/group) were exposed to 25% phthalic anhydride or 25 µL vehicle (4:1 (v/v) mixture of acetone and olive oil) via dermal application to the dorsum of both ears for 3 consecutive days. Three days after the last exposure (both inhalation and dermal), *ex vivo* cell proliferation and cytokine measurements were determined from mandibular and/or auricular lymph nodes (mandibular lymph nodes were not examined following dermal exposure). Arts et al. (2008) also provided quantitative histopathology of the respiratory tract, while De Jong et al. (2009) provided data on and production of cytokines (IL4, IL10 and IFN-γ) following dermal application of phthalic anhydride or inhalation.

Following inhalation exposure to phthalic anhydride, enlargement of the mandibular lymph nodes was observed, which corresponded with a significant increase in *ex vivo* cell proliferation in the mandibular lymph nodes (SI = 2.9 ± 0.52), which reflects a positive response in the LLNA assay. However, the relative lack of change in cell proliferation in the auricular lymph nodes (SI = 0.8 ± 0.009) reflects a negative response in the LLNA. The authors report the incidences of histopathologic lesions of the respiratory tract following inhalation exposure to aerosolized phthalic anhydride, including inflammatory lesions and squamous metaplasia/hyperplasia of the nasal cavity and larynx. However, the results for control animals are not provided, which severely impacts the ability to interpret these results. Following dermal exposure to phthalic anhydride (25%), a significant increase in *ex vivo* cell proliferation in the auricular lymph nodes was observed (SI = 93 ± 21), which reflects a positive result in the LLNA. Significant increases in the level of IL-4 as well as IFN-γ were observed following dermal application of phthalic anhydride, while slight but non-significant increases were observed following inhalation exposure (360 min) to phthalic anhydride. No significant changes were reported for IL-12 following inhalation or dermal application of phthalic anhydride; the data are presented as a qualitative statement of a null effect, which is a limitation that increases uncertainty in the dataset. Overall, data from these studies further support that phthalic anhydride is skin and respiratory sensitizer.

The study measured concentrations of phthalic anhydride as well as MMAD, however, values appear to reflect the average across a three-day period for the four experimental groups of animals combined (45, 90, 180, and 360 minutes). Although the authors report that the concentration was held constant, the doses of each exposure duration are not independently characterized which impacts the ability to compare them, which is crucial to understand given the variability in the respiratory LLNA results across exposure durations. Nevertheless, only the 360-minute group had an appropriate control so only the 360-minute group could be used to directly interpret the effect of phthalic anhydride exposure. Improper controls were also a limitation that impacted the histopathological dataset. Finally, the mediastinal lymph nodes are the primary draining lymph nodes for the respiratory tract, yet the authors evaluate the mandibular and auricular lymph nodes for the respiratory LLNA. The resulting uncertainty most likely results in a bias away from the null (*i.e.,* the effect is less likely to be detected).

Pulmonary Sensory Irritation Study of Phthalic Anhydride Dust in the Rat (IIT Research Institute, 1995)

Male SD rats (4 total) were exposed phthalic anhydride dust via head-only inhalation for at least 10 minutes (mean chamber phthalic anhydride concentration = 0.486 ± 0.109 mg chemical/L air; particle MMAD = 4.95 ± 1.87 µm; 21.7% of the mass was <3 µm and 92.7% of the mass was <9 µm). The exposure concentration of phthalic anhydride dust ranged from 0.442 to 0.645 mg/L across individual chambers, and the concentration of *o*-phthalic acid was reported to be nominal (*i.e.,* 0.93% of the total exposure concentration). The authors report that each rat acted as its own control. Pulmonary sensory irritation was quantified by measuring respiratory rates over a 3-minute period via plethysmograph; respiratory rates were calculated for 20-second intervals prior to the exposure, during, and after exposure. The study reported that a maximum attainable phthalic anhydride dust concentration of 0.574 mg/L did not produce pulmonary sensory irritation. Indeed, the authors indicate that there is no difference in the average respiratory rate during the pre-exposure period (132.1 breaths/minute), during (128.8 breaths/minute), or after exposure (130 breaths/minute); therefore, the test substance was considered to not have an effect on respiratory rate. There were several limitations of this study. The exact timing of the respiratory rate measurements was not provided, and no other endpoints were evaluated. Additionally, exposure to *o*-phthalic acid (0.93% of total collected sample) and phthalic anhydride vapor (0.05% of sample) also occurred; the mean *o*-phthalic acid concentration was measured at 0.00483 mg/L. The authors did not report results of statistical analyses, but sufficient data were

provided to conduct an independent statistical analysis (paired t-test). Study data are reported in Table_Apx A-2.

Table_Apx A-2. Respiratory Rates in Male Rats Exposed to Phthalic Anhydride (IIT Research Institute, 1995)

Animal Number	Respiratory Rate (breaths/minute) Mean ± SD		
	Pre-Exposure (Control)	During Exposure	Post-Exposure
885	116.1 ± 3.2[a]	111.6 ± 6.3	120.3 ±7.7
886	151.4 ± 9.4	143.7 ± 16.1	138.3 ± 5
887	118.7 ± 11.2	106.6 ± 3.9	118 ± 4.8
888	142.2 ± 5.8	153.1 ± 12	143.3 ± 5
Mean [b]	132.1 ±17.4	128.8 ± 23.1	130.0 ± 12.7
% of pre-exposure mean	100	97.5	98.4
Results of statistical analysis [c]	–	p = 0.551	p = 0.551

Source: Table 2 on page 8 of (IIT Research Institute, 1995)
[a] values are the mean respiratory rate ± standard deviation calculated during the measurement period (n = 6–11 20-second intervals per animal).
[b] Mean ± standard deviation of the average respiratory rate during the measurement period (n = 4 animals).
[c] Results of independent statistical analysis (paired two-tailed t-test) performed by EPA.

Respiratory Sensitization Study of Phthalic Anhydride: A Research Project (IIT Research Institute, 1996)

Another study by the IIT Research Institute (1996) described a two-part study in male and female SD rats. The first part examined exposure and challenge to phthalic anhydride, while the second part examined exposure and challenge with TMA to evaluate cross-reactivity. While part 1 is the focus of the results described below, discussion of results of part 2 where rats were exposed to phthalic anhydride and not subsequently challenged are also discussed.

In the first part, 10 male and 10 female rats were exposed via whole body inhalation to aerosolized particles of phthalic anhydride at a target concentration of 0.5 mg/m^3 for 6 hours/day for 5 days (TWA = 0.525 mg/m^3; range: 0.404–0.746 mg/m^3 across 5 days). The authors indicate that "respirable-sized" particles were aerosolized, but the authors do not provide quantitative measures of size (*i.e.,* MMAD) and distribution of the particles. The rats were rested for three weeks and then challenged with 0.481 mg/m^3 of phthalic anhydride for 6 hours. A control group of rats was also included that was not initially exposed to phthalic anhydride or challenged. Eighteen hours after challenge, lungs of all rats were examined for external hemorrhagic foci and lungs from three rats per group were examined microscopically for lesions. Levels of serum IgG specific to phthalic anhydride were evaluated in rats via ELISA.

Increased incidences of hemorrhagic lung foci were observed in rats exposed to and then challenged with phthalic anhydride (*i.e.,* 7/10 males; 3/10 females had ≥10 foci/lung compared to 0/20 animals in the control group). In parallel, increased numbers of phthalic-anhydride-specific IgG antibodies in serum were observed in rats exposed and challenged with phthalic anhydride compared to controls. Although female rats had a lower magnitude of increase in the number of foci compared to males, female rats had

significantly higher levels of IgG antibodies specific to phthalic anhydride. Lungs from three animals from each group were examined microscopically. In rats exposed to and challenged with phthalic anhydride, observations included alveolar hemorrhage (2/3 rats), parabronchial lymphoid hyperplasia (1/3 rats), and perivascular acute and chronic inflammation (1/3 rats). No microscopic lung lesions were observed in either control group of rats. No differences were observed in lung weight or lung volume. An abbreviated version of these studies is also found in a report by Amoco Chemical Company (1988).

There were key limitations in this study. First, the authors did not adequately characterize exposure to phthalic anhydride aerosols; no detail regarding the size or distribution of the particles was provided. Second, animals were exposed via whole-body inhalation, which introduces potential exposure via other routes beyond inhalation (*e.g.,* phthalic anhydride deposited on rodent skin reflects a dermal exposure, while deposits on fur may contribute oral exposure due to rodent behaviors such as grooming). Third, the authors report differences between the unexposed control rats and groups of rats exposed or challenged with phthalic anhydride. For the controls, insufficient detail was provided to ascertain whether the controls were air-chamber controls, or simply animals that remained in cages. The authors state (in regard to endpoint of body weight), "because controls were not fasted in the cage, [body weight in controls] could not be compared to [that of] the PA-exposed and challenged rats." This difference reflects a confounding variable in the study that may impact the ability to interpret results of the study beyond body weight. An additional limitation of the study was the insufficiency of sample size for one endpoint: microscopic evaluations in the lungs. Indeed, sample sizes of three animals total with mixed sexes (*i.e.,* 2 males and 1 female in phthalic anhydride and challenge group), are insufficient to conduct statistical analyses stratified by sex, which may impact the ability to interpret any sex-specific effects for this endpoint. However, despite the limitations, the study provides additional evidence that phthalic anhydride is a respiratory sensitizer. A LOAEC of 500 µg/m^3 was identified based on increased numbers of hemorrhagic foci per lung in rats.

Inhalation Challenge of Phthalic Anhydride Following Intradermal Injection in Guinea Pigs (Blaikie et al., 1995)

Blaikie et al. (1995) provide data on the allergenic potential of phthalic anhydride following sensitization with a single intradermal injection phthalic anhydride and inhalation challenge. The authors reported results of experiments conducted in two separate laboratories (*i.e.,* Laboratory 1 and 2). Passive cutaneous anaphylaxis (PCA) assay was used to evaluate elicitation of a type I hypersensitivity response to a hapten phthalic anhydride-GSA conjugate, and the serum titer that elicited a reaction with phthalic anhydride-GSA specific IgG1 antibodies was determined via ELISA. The phthalic anhydride-GSA conjugate dust used for inhalation challenges was generated using dry compressed air or argon gas to supply the atmosphere generation. Pulmonary responses were quantified after challenge, and were categorized for each individual animal based on the changes in respiratory rate. A "severe response" was characterized by a decrease in respiration rate to 70% or less of the normal background rate within the 15-minute challenge period; a "moderate response" was characterized as increase in respiration rate to 130% or more of the normal background rate within the 15-minute challenge period; "no response" was characterized as changes in the respiration rate within 71 to 129% of the normal background rate within the 15-minute challenge period.

In Laboratory 1, male and female Dunkin-Hartley guinea pigs (n = 8–12) were sensitized with phthalic anhydride (100 µL of 0, 0.03, 0.1, or 0.3%) phthalic anhydride in 6% acetone in corn oil vehicle). Eighteen days later (day 19), levels of serum IgG1 antibody were determined via ELISA and the extent of allergic reaction was determined via PCA. Animals were challenged with 44 mg/m^3 phthalic anhydride-GSA dust in argon or 52 mg/m^3 in dry air via inhalation on day 22 for 15 minutes.

In Laboratory 2, male and female Dunkin-Hartley guinea pigs were sensitized with phthalic anhydride (100 µL of 0 or 0.3%) phthalic anhydride in 6% acetone in corn oil vehicle. Eighteen days later (day 19), levels of serum IgG1 antibody were determined via ELISA and the extent of allergic reaction was determined via PCA. Animals were challenged with 11 to 29 mm/m^3 phthalic anhydride-GSA conjugate in argon or 9 to 48 mg/m^3 in dry air.

Results of serological analysis and pulmonary response after inhalation challenge in all experiments are presented in Table_Apx A-3.

Table_Apx A-3. Results of Serological Analysis and Pulmonary Response After Induction or Challenge with Phthalic Anhydride in Guinea Pigs (Blaikie et al., 1995)

Laboratory; Experiment	Induction [a]	Challenge	Serological Response [b]	Pulmonary Response (No-Moderate-Severe)
Laboratory 1; Experiment 1	0, 0.03, 0.1, or 0.3% phthalic anhydride in 6% acetone in corn oil vehicle	0 or 11–29 mg/m³ phthalic anhydride (MMAD: 3.79–4.81 µm) in argon	PCA: 0/8, 2/8, 6/8, 7/8	6-1-1/8; 6-1-1/8; 5-1-1/8; 4-0-4/8
Laboratory 1; Experiment 2	0, 0.03, 0.1, or 0.3% phthalic anhydride in 6% acetone in corn oil vehicle	0 or 9–48 mg/m³ of phthalic anhydride (MMAD: 0.61–18.02 µm) in dry air	PCA: 0/8; 7/7; 8/8; 8/8	8-0-0/; 6-0-1/7; 7-0-1/8; 5-0-3/8
Laboratory 2; Experiment 1	0 or 0.3 % in acetone in corn oil	0 or 44 mg/m³ of phthalic anhydride (MMAD: 5.9–1.6 µm) in air	PCA: 1/8; 12/12;	8/8; 12-0-0/12
Laboratory 2; Experiment 2	0 or 0.3 % in acetone in corn oil	0, 52 mg/m³ of phthalic anhydride (MMAD: 4.7–1.6 µm) in air	Not tested	6-0-1/7, 4-1-7/12

MMAD = mass median aerodynamic diameter; PCA = passive cutaneous anaphylaxis assay
[a] Exposure (induction and challenge) to phthalic anhydride-GSA conjugate
[b] Serological response (no. of animals with positive test) determined via PCA or ELISA from blood sample collected 18 days after intradermal injection with phthalic anhydride.
[c] Pulmonary response (no. of animals with "no response" "moderate response" or "severe response") determined after challenge with to phthalic anhydride-GSA conjugate as indicated 21 days after intradermal injection with phthalic anhydride.

Pulmonary responses were not observed following challenge with 44 mg/m^3 phthalic anhydride-GSA dust in Laboratory 1, which the authors attributed to rapid oxidation of the test substance. Therefore, they repeated the experiment with dry air or argon gas. Positive pulmonary responses were observed in animals challenged with phthalic anhydride in dry air via inhalation. A phthalic anhydride-specific pulmonary response was observed in animals challenged with 11 to 29 mm/m^3 phthalic anhydride-GSA conjugate in argon (4 of 8 animals had a "severe" response) as well as animals challenged with 9 to 48 mg/m^3 in dry air (3 of 8 animals had a "severe" response). Except for the first experiment that did not use dry air or argon as a carrier gas, both laboratories and experiments reported positive results in the PCA and increased serum levels of anti-phthalic anhydride-GSA IgG1 antibody (via ELISA) in animals challenged with phthalic anhydride-GSA conjugates. Collectively, these data provide additional support that phthalic anhydride is a sensitizer.

Limitations of the data include that the results for the pulmonary response are presented as subjective incidences of a categorical response (no effect, moderate, severe), and results of statistical analyses are not provided. Another limitation of the study is that the MMAD reported by the authors reflects that of the phthalic anhydride-GSA conjugate, not phthalic anhydride alone. The molecular weight of phthalic anhydride is 148 g/mol, while the molecular weight of the phthalic anhydride-GSA conjugate is

estimated to be approximately 68,400 g/mol. It is likely that steric hindrance of the GSA albumin conjugate influences its interactions at the molecular level, which in turn may affect the cellular immune response and contribute uncertainty to the dataset. The use of the GSA-phthalic anhydride conjugate limits the ability to interpret the results relating to immune responses.

A Method for Measuring Mouse Respiratory Allergic Reaction to Low-Dose Chemical Exposure to Allergens (Fukuyama et al., 2010)

Fukuyama et al. (2010) applied 25 µL of vehicle (4:1 acetone:olive oil) or 0.3% phthalic anhydride to the dorsum of both ears of young (7 weeks) female BALB/c mice on days 1 to 3, 8 to 10, and 15 to 17. Following a two-week rest period, mice were anesthetized and were challenged intratracheally with either 50 µL of vehicle or 0.03% phthalic anhydride (*i.e.,* on study day 31). Three experimental groups were included in the study design, including (1) induction with vehicle plus challenge with vehicle (-/-); (2) induction with phthalic anhydride plus challenge with vehicle (+/-); and (3) induction with phthalic anhydride plus challenge with phthalic anhydride (+/+). On the day after the challenge (Day 32), mice were sacrificed. Animals induced and challenged with phthalic anhydride (+/+ group) had total IgE and IgG1 levels that were significantly increased in serum and bronchoalveolar lavage fluid (BALF) compared to control animals. Additionally, animals induced and challenged with phthalic anhydride had (1) increased total cell counts, eosinophils, and neutrophils in BALF; (2) increased chemokine and cytokine levels (*i.e.,* IL-6, TNF- α, MCP-1, EOTAXIN-1, MIP1-β) in BALF; and (3) increased expression of CCR3 mRNA in BALF. Finally, lung-associated lymph nodes (precise lymph nodes excised not stated) were excised and lymphocyte populations were characterized. Compared to controls, animals induced and challenged with phthalic anhydride (+/+ group) had increased total cell counts, increased numbers of IgE positive B cells, and increased counts of MHC class II positive B cells in lung-associated lymph nodes. *Ex situ* cytokine (*i.e.,* IL-4, IL-5, IL-13, IFN-γ) production was also elevated in lung-associated lymph node supernatant collected from animals induced and challenged with phthalic anhydride. Gene expression of Th2 type allergy-related genes (GATA-3, STAT6, CCR4) were also evaluated in cells isolated from lung-associated lymph nodes; however, gene expression was not significantly altered by treatment with phthalic anhydride.

A.3.1 Eye Irritation

Phthalic anhydride is classified (GHS) as Eye Dam. 1 (H318: Causes serious eye damage) in the EU (https://chem.echa.europa.eu/100.001.461/overview, accessed February 2, 2026). Evidence of eye irritation comes from several occupational exposure studies of humans and studies of experimental animal models.

Nielsen et al. (1988) reported that irritation of the eyes included conjunctivitis (46% of workers) and rhinitis (40% of workers). In the heavily exposed workers, 69% suffered from conjunctivitis and/or rhinitis. Subsequently, Nielsen et al., (1991) found that more work-related symptoms were seen in the eyes of exposed workers than controls.

Original copies of eye irritation studies of laboratory animals were not reasonably available to EPA; however, study summaries are reported by OECD (2005) and available information is briefly summarized below.

In the first study, which was assigned a reliability score of 2 (reliable with restrictions) by OECD (2005), 50 mg of solid phthalic anhydride was applied to the conjunctival sac of two New Zealand White rabbits (1/sex) and ocular irritation was scored according to the Draize method after 1 hour, and 1, 2, and 7 days. Observed effects included transitional slight cloudiness of the cornea; conjunctiva redness; conjunctiva swelling, and lacrimation. All effects were reversible within the 7-day period,

except for conjunctiva redness which persisted on study day 7 (score 1 at day 7). Under the conditions of the study, phthalic anhydride was considered to have irreversible effects on the eye.

In a second study, which was assigned a reliability score of 4 (not assignable, due to an unreliable test institute [Industrial Biotest Laboratories]) by OECD (2005), 100 mg of dry phthalic anhydride powder was applied to the conjunctival sac of one eye of six rabbits. Ocular irritation was scored 24-, 48-, and 72-hours post application according to the standard U.S. method (Code of Federal Registrations, 1981). Mean irritancy scores of 71/110, 77.3/110, and 81/110 were obtained at 24, 48, and 72 hours, respectively. Under the conditions of the study, phthalic anhydride was considered an eye irritant.

Appendix B ANALOG IDENTIFICATION

EPA conducted a search for potential analogs with inhalation exposure data suitable for dose-response assessment to supplement its assessment of phthalic anhydride. This appendix describes EPA's approach for analog identification.

EPA has preliminarily identified trimellitic anhydride (TMA) as an analog for phthalic anhydride based on structural similarity (Appendix B.1); similar physical, chemical, and fate properties (Appendix B.2); and toxicological similarity (Appendix B.3). Reasonable available laboratory animal inhalation toxicity studies of TMA are discussed in Appendix B.4, while reasonably available human evidence for TMA is discussed in Appendix B.5, and EPA's weight of scientific evidence conclusions are discussed further in Appendix B.6.

B.1 Identification of Candidate Analogs Based on Structural Similarity and Hazard Information

Structural similarity between phthalic anhydride and candidate analogs was assessed using the Analog Identification Methodology (AIM) program (*i.e.,* a NAM identified in the TSCA section 4(h)(2)(C) List of NAMs, and two additional EPA products, including the search module within the Cheminformatics Modules (https://www.epa.gov/comptox-tools/cheminformatics; accessed March 26, 2026) and the Generalized Read-Across (GenRA) tool (https://comptox.epa.gov/genra; accessed March 26, 2026). AIM analysis was performed on the CBI-side and analogs were described as first or second pass. Tanimoto scores were obtained in the Cheminformatics Search Module using a similar analysis with CASRN 88-44-9 for phthalic anhydride. The phthalic anhydride SMILES (O=C1OC(=O)C2=C1C=CC=C2) was user-defined in GenRA (Version 3.3.1) to generate chemical Morgan fingerprints for phthalic anhydride (limit of 100 analogs, no ToxRef filter).

AIM first and second pass analogs were compiled along with the Cheminformatics search module results with Tanimoto coefficients greater than or equal to 0.9 and with the top 100 analogs identified via GenRA. As can be seen from Table_Apx B-1, AIM returned two potential analogs, including one first-pass (exact match) and one second pass analog. Cheminformatics returned 154 potential analogs with Tanimoto coefficients between 0.90 and 1.00. GenRA was limited to 100 analogs (no ToxRef Filter). As can be seen from Table_Apx B-1, of the identified potential analogs, 1 was identified using all three tools (AIM, GenRA and Cheminformatics), while 14 were identified by both GenRA and Cheminformatics. The remaining chemicals potential analogs were each identified using a single tool.

Next, EPA screened candidate analogs in Table_Apx B-1 for available hazard data using EPA's CompTox Dashboard (v.2.5.3, accessed April 14, 2025). Briefly, information in the 'Hazard Data' tab was reviewed for each candidate analog in Table_Apx B-1. Of the candidate analogs identified, 23 had hazard data available (Table_Apx B-1). The majority of these chemicals (18 of 23) only had genotoxicity and/or acute toxicity six-pack data reported (acute toxicity testing, skin/eye irritation, skin sensitization), which would not address identified limitations in the phthalic anhydride database (*i.e.,* limited dose-response studies for the inhalation route). Of the remaining five chemicals, four had repeated dose inhalation data reported (*i.e.,* TMA; diethyl phthalate; anthraquinone; 1,2-Benzenedicarboxylic acid, di-C6-10-alkyl esters). Of these four, EPA selected TMA for subsequent comparisons of physical and chemical and fate properties (Appendix B.2) and a comparison of toxicological similarity (Appendix B.3). EPA selected TMA because, like phthalic anhydride, TMA is also a dermal and respiratory sensitizer, and both phthalic anhydride and TMA fall within the same chemical category (*i.e.,* both are carboxylic acid anhydrides) (Health Canada, 2019; U.S. EPA, 2010).

Table_Apx B-1. Summary of Candidate Analogs for Phthalic Anhydride Based on Structural Similarity

DTXCID	CASRN	Name	Similarity (Cheminform.)	Similarity (GenRA)	AIM	Hazard Data Available in CompTox?	If Yes, Type of Data
DTXCID906235	552-30-7	Trimellitic anhydride	1.0	–	–	Yes	Repeated dose (oral, dermal, inhalation); Acute (oral) toxicity Genotoxicity; Skin Irritation; Eye irritation; Skin sensitization
DTXCID701526672	68515-51-5	1,2-Benzenedicarboxylic acid, di-C6-10-alkyl esters	0.9444444	–	–	Yes	Repeated dose (inhalation); Genotoxicity; Skin irritation; Eye irritation
DTXCID9095	84-65-1	Anthraquinone	–	0.400000006	–	Yes	Repeated dose (oral, inhalation); Genotoxicity; Skin sensitization; Eye irritation; skin irritation
DTXCID901780	84-66-2	Diethyl phthalate	0.9444444	–	–	Yes	Repeated dose (oral, dermal, inhalation); Acute toxicity (oral, dermal, inhalation); Genotoxicity; Skin sensitization; skin irritation; eye irritation
DTXCID70810032	17369-59-4	3-Propylidenephthalide	–	0.394736856	–	Yes	Repeated dose (oral); Acute (oral) toxicity; Genotoxicity; Skin sensitization

DTXCID	CASRN	Name	Similarity (Cheminform.)	Similarity (GenRA)	AIM	Hazard Data Available in CompTox?	If Yes, Type of Data
DTXCID7031167	87-41-2	Phthalide	0.9722222	–	–	Yes	Acute (oral) toxicity; Genotoxicity
DTXCID6024886	2420-87-3	5,5'-Bi-2-benzofuran-1,1',3,3'-tetrone	0.9230769	0.424242437	–	Yes	Genotoxicity
DTXCID901390629	43011-20-7	1,3-Dioxo-1,3-dihydro-2-benzofuran-5-carboxylic acid--ethane-1,2-diol (1/1)	1.0	–	–	Yes	Genotoxicity
DTXCID9092040	1732-96-3	Ethylene bis(1,3-dihydro-1,3-dioxoisobenzofuran-5-carboxylate)	0.972973	–	–	Yes	Genotoxicity
DTXCID001401249	58249-83-5	2-[(Benzoyloxy)methyl]benzoic acid	0.9444444	–	–	Yes	Skin sensitization
DTXCID401026273	67846-10-0	1,2,4-Benzenetricarboxylic acid, 1,2-bis(phenylmethyl) ester	0.9444444	–	–	Yes	Skin sensitization
DTXCID7023938	2528-16-7	Monobenzyl phthalate	0.9444444	–	–	Yes	Skin sensitization
DTXCID8092471	1754-55-8	Ethyl 2,4,6-trimethylbenzoate	0.9444444	–	–	Yes	Skin Sensitization
DTXCID201408558	63948-88-9	1,3-Dioxo-1,3-dihydro-2-benzofuran-5-carboxylic acid--propane-1,2-diol (1/1)	0.9230769	–	–	Yes	Genotoxicity; Skin sensitization; skin irritation; eye irritation
DTXCID805094	117-82-8	Di(2-methoxyethyl) phthalate	0.9189189	–	–	Yes	Acute toxicity (oral, dermal); Genotoxicity
DTXCID901371118	2055-00-7	2,2'-Ethane-1,2-diyl dimethyl dibenzene-1,2-dicarboxylate	0.9189189	–	–	Yes	Skin sensitization
DTXCID1031127	81-30-1	Naphthalenetetracarboxylic dianhydride	–	0.444444448	–	Yes	Acute (oral) toxicity
DTXCID70157833	86-90-8	4-Bromophthalic anhydride	–	0.400000006	–	Yes	Skin sensitization
DTXCID9074484	118-45-6	4-Chlorophthalic anhydride	–	0.400000006	–	Yes	Genotoxicity; Skin sensitization; Eye irritation; skin irritation
DTXCID606505	81-84-5	1H,3H-Naphtho(1,8-cd)pyran-1,3-dione	–	0.393939406	–	Yes	Acute (oral, dermal) toxicity; Skin sensitization

DTXCID	CASRN	Name	Similarity (Cheminform.)	Similarity (GenRA)	AIM	Hazard Data Available in CompTox?	If Yes, Type of Data
DTXCID40108872	32703-79-0	4-tert-Butylphthalic anhydride	–	0.378378391	–	Yes	Skin sensitization
DTXCID9024859	1823-59-2	5,5'-Oxybis(2-benzofuran-1,3-dione)	–	0.378378391	–	Yes	Genotoxicity
DTXCID9037296	2540-99-0	1,3-Isobenzofurandione, 5,5'-sulfonylbis-	–	0.378378391	–	Yes	Genotoxicity Skin sensitization
DTXCID6047078	716-39-2	Naphtho[2,3-c]furan-1,3-dione	0.94736844	0.419354826	2nd Pass	No	
DTXCID90534526	75935-32-9	(~2~H_4_)-2-Benzofuran-1,3-dione	1.0	0.413793117	–	No	
DTXCID90521491	5981-12-4	Triphenyleno[2,3-c]furan-10,12-dione	0.94736844	0.382352948	–	No	
DTXCID80276423	955-16-8	3,4-Biphenyldicarboxylic anhydride	0.9230769	0.375	–	No	
DTXCID801414812	68155-85-1	2-Benzofuran-1,3-dione--propane-1,3-diol (1/1)	0.94736844	0.730769217	–	No	
DTXCID70405947	6812-14-2	2,3-Anthracenedicarboxylic Anhydride	0.94736844	0.371428579	–	No	
DTXCID501763836	21161-08-0	3,4,6,7,9,10,12,13-Octahydro-2,5,8,11,14-benzopentaoxacycloheptadecin-1,15-dione	0.9189189	0.371428579	–	No	
DTXCID307805	19438-61-0	4-Methylphthalic anhydride	1.0	0.400000006	–	No	
DTXCID301763961	16709-52-7	7,8,17,18-Tetrahydrodibenzo[f,n][1,4,9,12]tetraoxacyclohexadecine-5,10,15,20-tetrone	0.9189189	0.448275864	–	No	
DTXCID3030260	13988-26-6	3,4,6,7-Tetrahydro-2,5,8-benzotrioxacycloundecin-1,9-dione	0.9189189	0.382352948	–	No	
DTXCID30117276	4196-98-9	Ethylene phthalate	0.9189189	0.448275864	–	No	
DTXCID201381906	31976-47-3	2-Benzofuran-1,3-dione--propane-1,2-diol (1/1)	0.9230769	0.655172408	–	No	
DTXCID10505793	106070-55-7	5,5'-(1,4-Phenylene)di(2-benzofuran-1,3-dione)	0.9230769	0.388888896	–	No	
DTXCID101418075	7073-35-0	1,3-Dioxo-1,3-dihydro-2-benzofuran-5-carbaldehyde	1.0	0.378378391	–	No	
–	25038-84-0	–	–	–	1st Pass (exact Match)	No	
DTXCID0040593	4792-30-7	1,3-Isobenzofurandione, 4-methyl-	1.0	–	–	No	
DTXCID201430440	83536-59-8	4,5-Dimethyl-2-benzofuran-1,3-dione	1.0	–	–	No	
DTXCID301402810	5999-20-2	5,6-Dimethyl-2-benzofuran-1,3-dione	1.0	–	–	No	
DTXCID30409885	65399-04-4	4-Isobenzofurancarboxylic acid, 1,3-dihydro-1,3-dioxo-, methyl ester	1.0	–	–	No	
DTXCID801350860	116211-88-2	4-(Hydroxymethyl)-2-benzofuran-1,3-dione	1.0	–	–	No	
DTXCID90125554	5463-50-3	4,7-Dimethyl-1,3-isobenzofurandione	1.0	–	–	No	
DTXCID901430441	83536-60-1	4,5,6-Trimethyl-2-benzofuran-1,3-dione	1.0	–	–	No	
DTXCID901763595	57913-85-6	Dimethyl 1,3-dioxo-1,3-dihydro-2-benzofuran-5,6-dicarboxylate	1.0	–	–	No	

DTXCID	CASRN	Name	Similarity (Cheminform.)	Similarity (GenRA)	AIM	Hazard Data Available in CompTox?	If Yes, Type of Data
DTXCID9037907	2902-64-9	5-Isobenzofurancarboxylic acid, 1,3-dihydro-1,3-dioxo-, methyl ester	1.0	–	–	No	
DTXCID901397675		Unnamed chemical	–	0.655172408	–	No	
DTXCID701383771		Unnamed chemical	–	0.633333325	–	No	
DTXCID401380904		Unnamed chemical	–	0.612903237	–	No	
DTXCID801382245		Unnamed chemical	–	0.612903237	–	No	
DTXCID20237210	6328-17-2	1H-2-Benzopyran-1,3,4-trione	–	0.586206913	–	No	
DTXCID301414524	67953-40-6	3-Hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid--2-benzofuran-1,3-dione (1/1)	–	0.575757563	–	No	
DTXCID101727870	4440-44-2	1H-Cyclohepta[c]furan-1,3,6-trione	–	0.518518507	–	No	
DTXCID20307644	19357-64-3	.DELTA.3,3'-Biphthalide	–	0.517241359	–	No	
DTXCID301067858		Unnamed chemical	–	0.517241359	–	No	
DTXCID80307645	482-23-5	(3Z)-3-(3-Oxo-2-benzofuran-1(3H)-ylidene)-2-benzofuran-1(3H)-one	–	0.517241359	–	No	
DTXCID301402303	59480-26-1	1,3-Dioxo-1,3-dihydro-2-benzofuran-5-carboxylic acid--2,2'-oxydi(ethan-1-ol) (1/1)	0.972973	–	–	No	
DTXCID701556487	14513-41-8	5,6-Isobenzofurandicarboxylic acid, 1,3-dihydro-1,3-dioxo-, 5,5′-(1,2-ethanediyl) ester	0.972973	–	–	No	
DTXCID0066959	101976-08-3	Phthalaldehydic anhydride	0.9722222	–	–	No	
DTXCID10119839	4792-29-4	1,3-Dihydro-1-oxoisobenzofuran-5-carboxylic acid	0.9722222	–	–	No	
DTXCID10430910	54401-64-8	1(3H)-Isobenzofuranone, 5,7-dimethyl-	0.9722222	–	–	No	
DTXCID20100450	23405-31-4	Ethyl 1,3-dihydro-1-oxoisobenzofuran-5-carboxylate	0.9722222	–	–	No	
DTXCID301653380	24129-05-3	Methyl 1,3-dihydro-3-oxo-4-isobenzofurancarboxylate	0.9722222	–	–	No	
DTXCID30293781	54598-91-3	4,7-Dimethyl-3H-isobenzofuran-1-one	0.9722222	–	–	No	
DTXCID30299667	72985-23-0	6-Methylphthalide	0.9722222	–	–	No	
DTXCID30512621	51648-98-7	5,6-Dimethyl-2-benzofuran-1(3H)-one	0.9722222	–	–	No	
DTXCID401666702	4743-61-7	1,3-Dihydro-3-oxo-5-isobenzofurancarboxylic acid	0.9722222	–	–	No	
DTXCID40514292	4792-27-2	1-Oxo-1,3-dihydro-2-benzofuran-4-carboxylic acid	0.9722222	–	–	No	
DTXCID50583459	23405-32-5	Methyl 1-oxo-1,3-dihydro-2-benzofuran-5-carboxylate	0.9722222	–	–	No	
DTXCID60124958	54120-64-8	5-Methyl-3H-2-benzofuran-1-one	0.9722222	–	–	No	
DTXCID70299666	2211-83-8	4-methyl-2-benzofuran-1(3H)-one	0.9722222	–	–	No	
DTXCID70542129	452978-21-1	6-(Hydroxymethyl)-2-benzofuran-1(3H)-one	0.9722222	–	–	No	
DTXCID70801900	51648-99-8	6-Methyl-1-oxo-1,3-dihydro-2-benzofuran-5-carboxylic acid	0.9722222	–	–	No	
DTXCID80132010	607-86-3	2-Methylbenzoic acid anhydride	0.9722222	–	–	No	
DTXCID80273735	5745-51-7	mesitoic anhydride	0.9722222	–	–	No	
DTXCID80382997	333333-34-9	1-oxo-1,3-dihydroisobenzofuran-5-carbaldehyde	0.9722222	–	–	No	

DTXCID	CASRN	Name	Similarity (Cheminform.)	Similarity (GenRA)	AIM	Hazard Data Available in CompTox?	If Yes, Type of Data
DTXCID901668622	4792-28-3	1,3-Dihydro-3-oxo-4-isobenzofurancarboxylic acid	0.9722222	–	–	No	
DTXCID90299668	2211-84-9	7-methyl-2-benzofuran-1(3H)-one	0.9722222	–	–	No	
DTXCID90449469	65006-89-5	5-(Hydroxymethyl)-2-benzofuran-1(3H)-one	0.9722222	–	–	No	
DTXCID00445396	5665-50-9	Phenanthro[2,3-c]furan-8,10-dione	0.94736844	–	–	No	
DTXCID00657356	26363-45-1	1,3-Dioxo-1,3-dihydronaphtho[2,3-c]furan-6,7-dicarboxylic acid	0.94736844	–	–	No	
DTXCID001518307	86569-88-2	Ethyl 2,3,5-trichlorobenzoate	0.9444444	–	–	No	
DTXCID00285552	36596-67-5	Ethyl 2,6-dimethylbenzoate	0.9444444	–	–	No	
DTXCID00440386	4909-77-7	Benzyl 2,4,6-trimethylbenzoate	0.9444444	–	–	No	
DTXCID00793460	920751-82-2	1,3-Diethyl 2,5-dimethyl benzene-1,2,3,5-tetracarboxylate	0.9444444	–	–	No	
DTXCID10292217	55133-99-8	Benzoic acid, 2-methyl-, (2-methylphenyl)methyl ester	0.9444444	–	–	No	
DTXCID1084504	14230-18-3	1,2,4-Benzenetricarboxylic acid, triethyl ester	0.9444444	–	–	No	
DTXCID20109625	33499-42-2	Ethyl 2,4-dimethylbenzoate	0.9444444	–	–	No	
DTXCID201644708	1217862-85-5	4-[(Acetyloxy)methyl]-2,3,5,6-tetramethylbenzaldehyde	0.9444444	–	–	No	
DTXCID2020711	87-24-1	Ethyl 2-methylbenzoate	0.9444444	–	–	No	
DTXCID20400003	34046-43-0	Ethyl 2-formylbenzoate	0.9444444	–	–	No	
DTXCID20534796	93952-12-6	Diethyl (~2~H_4_)benzene-1,2-dicarboxylate	0.9444444	–	–	No	
DTXCID301518306	86569-86-0	Ethyl 2,4,5-trichlorobenzoate	0.9444444	–	–	No	
DTXCID301735388	96259-60-8	Ethyl 2-(ethoxymethyl)benzoate	0.9444444	–	–	No	
DTXCID301775649	67157-60-2	Benzyl 2-methylbenzoate	0.9444444	–	–	No	
DTXCID30712705	105702-53-2	4-(Ethoxycarbonyl)benzene-1,3-dicarboxylate	0.9444444	–	–	No	
DTXCID30806956	408536-82-3	Diethyl 2,6-dimethylbenzene-1,4-dicarboxylate	0.9444444	–	–	No	
DTXCID30818291	523-31-9	Dibenzyl phthalate	0.9444444	–	–	No	
DTXCID401023786	6862-68-6	1,2,4,5-Benzenetetracarboxylic acid, 1,5-diethyl ester	0.9444444	–	–	No	
DTXCID40111467	35461-75-7	2-(Acetoxymethyl)benzoic acid	0.9444444	–	–	No	
DTXCID40293015	55000-48-1	2,4-Dimethylbenzyl 2,5-dimethylbenzoate	0.9444444	–	–	No	
DTXCID501024363	16927-06-3	1,2,4,5-Benzenetetracarboxylic acid, 1,4-diethyl ester	0.9444444	–	–	No	
DTXCID50110108	34006-77-4	Methyl ethyl phthalate	0.9444444	–	–	No	
DTXCID501284767	NOCAS_892730	2-(Ethoxycarbonyl)(O-~2~H)benzoic acid	0.9444444	–	–	No	
DTXCID50276394	55352-35-7	ethyl 2,3,5,6-tetramethylbenzoate	0.9444444	–	–	No	
DTXCID50697520	1015854-62-2	Dibenzyl (~2~H_4_)benzene-1,2-dicarboxylate	0.9444444	–	–	No	
DTXCID50736049	4619-49-2	2-([(2-Methylphenyl)methoxy]carbonyl)benzoate	0.9444444	–	–	No	
DTXCID601508609	68988-18-1	1,2-Benzenedicarboxylic acid, di-C4-13-alkyl esters	0.9444444	–	–	No	
DTXCID60509746	33499-43-3	Ethyl 2,5-dimethylbenzoate	0.9444444	–	–	No	

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DTXCID60793461	920751-81-1	2,5-Diethyl 1,3-dimethyl benzene-1,2,3,5-tetracarboxylate	0.9444444	–	–	No	
DTXCID70127410	56366-05-3	Benzene-1,2,3-tricarboxylic acid, triethyl ester	0.9444444	–	–	No	
DTXCID701664381	107170-82-1	1,3-Diethyl 2,5-dimethyl-1,3-benzenedicarboxylate	0.9444444	–	–	No	
DTXCID701763444	99873-99-1	Bis(1,1,2,2,2-pentadeuterioethyl) 3,4,5,6-tetradeuteriobenzene-1,2-dicarboxylate	0.9444444	–	–	No	
DTXCID7031294	2306-33-4	Monoethyl phthalate	0.9444444	–	–	No	
DTXCID70346205	140410-09-9	1,3-Benzenedicarboxylic acid, 4,6-dimethyl-, diethyl ester	0.9444444	–	–	No	
DTXCID70808873	1219806-03-7	2-(Ethoxycarbonyl)(~2~H_4_)benzoic acid	0.9444444	–	–	No	
DTXCID801284942	NOCAS_892905	2-[(Benzyloxy)carbonyl](O-~2~H)benzoic acid	0.9444444	–	–	No	
DTXCID801684293	104216-92-4	Benzoic acid, 2-(methoxymethyl)-, ethyl ester	0.9444444	–	–	No	
DTXCID80303561	137380-48-4	3-FORMYL-2,4,6-TRIMETHYLBENZYL ACETATE	0.9444444	–	–	No	
DTXCID80414247	365534-57-2	BENZYL 2-HYDROXYMETHYLBENZOATE	0.9444444	–	–	No	
DTXCID80808869	478954-83-5	2-[(Benzyloxy)carbonyl](~2~H_4_)benzoic acid	0.9444444	–	–	No	
DTXCID90139035	6634-01-1	Tetraethylpyromellitate	0.9444444	–	–	No	
DTXCID901629664	56863-79-7	4-Ethyl 2-methyl 3,5-dimethyl-1,2,4-benzenetricarboxylate	0.9444444	–	–	No	
DTXCID90240195	113674-51-4	benzyl 2-formylbenzoate	0.9444444	–	–	No	
DTXCID90583534	91034-37-6	Benzyl ethyl benzene-1,2-dicarboxylate	0.9444444	–	–	No	
DTXCID00773247	6709-19-9	Chryseno[5,6-c]furan-1,3-dione	0.9230769	–	–	No	
DTXCID20124090	5343-99-7	1,2-Naphthalenedicarboxylic anhydride	0.9230769	–	–	No	
DTXCID20275879	7499-48-1	NSC407661	0.9230769	–	–	No	
DTXCID20672768	4393-77-5	(3bE,5E,7Z,9E,11Z,11bZ,13E,15Z,17E,19E)-Dicyclodeca[3,4:5,6]benzo[1,2-c]furan-1,3-dione	0.9230769	–	–	No	
DTXCID30234119	2510-53-4	phenanthro[9,10-c]furan-1,3-dione	0.9230769	–	–	No	
DTXCID401701643	40682-58-4	4,9-Dimethylnaphtho[2,3-c]furan-1,3-dione	0.9230769	–	–	No	
DTXCID40273251	5723-54-6	Phenanthro[3,4-c]furan-1,3-dione	0.9230769	–	–	No	
DTXCID40276368	36440-65-0	8-methylbenzo[e][2]benzofuran-1,3-dione	0.9230769	–	–	No	
DTXCID501707701	51557-30-3	Benzo[8,9]chryseno[5,6-c]furan-5,7-dione	0.9230769	–	–	No	
DTXCID50276253	6709-40-6	Benzo[3,4]phenanthro[1,2-c]furan-5,7-dione	0.9230769	–	–	No	
DTXCID50402757	113837-02-8	1,3-Isobenzofurandione, 5,5'-(1,3-phenylene)bis-	0.9230769	–	–	No	
DTXCID601516909	52005-46-6	Dichlorophthalic anhydride	0.9230769	–	–	No	
DTXCID90352404	4711-50-6	naphtho[2,3-c]furan-1(3H)-one	0.92105263	–	–	No	
DTXCID001415092	68389-55-9	Benzene-1,2-dicarboxylic acid--2-[2-(2-hydroxyethoxy)ethoxy]ethyl benzoate (1/1)	0.9189189	–	–	No	
DTXCID001415204	68439-19-0	2-((2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy)carbonyl)benzoic acid	0.9189189	–	–	No	

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DTXCID00720655	140448-92-6	2-[2-(Acetyloxy)ethoxy]ethyl 2-formylbenzoate	0.9189189	–	–	No	
DTXCID101023294	2202-98-4	1,2-Benzenedicarboxylic acid, 1-[2-(2-hydroxyethoxy)ethyl] ester	0.9189189	–	–	No	
DTXCID1048382	84-73-1	Bis(2-hydroxyethyl) phthalate	0.9189189	–	–	No	
DTXCID1092862	17773-18-1	Tris(2-hydroxyethyl) benzene-1,2,4-tricarboxylate	0.9189189	–	–	No	
DTXCID201423005	7447-67-8	2,2'-(Oxybis[(ethane-2,1-diyl)oxycarbonyl])dibenzoic acid	0.9189189	–	–	No	
DTXCID20551887	603109-82-6	4-[(2-Hydroxyethoxy)carbonyl]benzene-1,2-dicarboxylate	0.9189189	–	–	No	
DTXCID20739709	52642-34-9	1,6-Dioxo-1,3,4,6-tetrahydro-2,5-benzodioxocine-8-carboxylate	0.9189189	–	–	No	
DTXCID301024331	16501-01-2	1,2-Benzenedicarboxylic acid, 1-(2-methoxyethyl) ester	0.9189189	–	–	No	
DTXCID301367381	17689-42-8	2-[(2-Hydroxyethoxy)carbonyl]benzoic acid	0.9189189	–	–	No	
DTXCID301537217	170303-76-1	7,8,10,11,13,14,23,24,26,27,29,30-Dodecahydrodibenz[l,z][1,4,7,10,15,18,21,24]octaoxacyclooctacosin-5,16,21,32-tetrone	0.9189189	–	–	No	
DTXCID3074151	117-85-1	Bis(2-(2-ethoxyethoxy)ethyl) phthalate	0.9189189	–	–	No	
DTXCID401517737	7517-39-7	2,2'-[1,2-Ethanediylbis(oxycarbonyl)]dibenzoic acid	0.9189189	–	–	No	
DTXCID401566465	97546-24-2	1,2-Benzenedicarboxylic acid, 1,1′-[1,2-ethanediylbis(oxy-2,1-ethanediyl)] ester	0.9189189	–	–	No	
DTXCID501764010	1398065-54-7	Bis(2-methoxyethyl) 3,4,5,6-tetradeuteriobenzene-1,2-dicarboxylate	0.9189189	–	–	No	
DTXCID50584340	90327-13-2	2-Ethoxyethyl 2-methylbenzoate	0.9189189	–	–	No	
DTXCID601551353	1207594-58-8	Poly(oxy-1,2-ethanediyloxycarbonyl-1,2-phenylenecarbonyl), α-acetyl-ω-[2-(acetyloxy)ethoxy]-	0.9189189	–	–	No	
DTXCID60231813	6297-46-7	2-methoxyethyl 2-methylbenzoate	0.9189189	–	–	No	
DTXCID6042818	605-54-9	1,2-Benzenedicarboxylic acid, bis(2-ethoxyethyl) ester	0.9189189	–	–	No	
DTXCID701565066	15268-12-9	1,4-Benzenedicarboxylic acid, 2,3,5,6-tetramethyl-, 1,4-bis(2-hydroxyethyl) ester	0.9189189	–	–	No	
DTXCID701763787	31937-98-1	Bis[2-(2-hydroxyethoxy)ethyl] benzene-1,2-dicarboxylate	0.9189189	–	–	No	
DTXCID701763999	1398066-12-0	Phthalic acid bis-2-ethoxyethyl ester D4	0.9189189	–	–	No	
DTXCID70241781	16672-71-2	Bis[2-(2-methoxyethoxy)ethyl] benzene-1,2-dicarboxylate	0.9189189	–	–	No	
DTXCID80487254	95241-37-5	Tris(2-ethoxyethyl) benzene-1,2,4-tricarboxylate	0.9189189	–	–	No	
DTXCID90241465	36339-61-4	2-methoxyethyl methyl benzene-1,2-dicarboxylate	0.9189189	–	–	No	
DTXCID10292959	55000-46-9	2,4-Dimethylbenzyl 3,5-dimethylbenzoate	0.9166667	–	–	No	
DTXCID70241766	38418-11-0	2-Methylbenzyl benzoate	0.9166667	–	–	No	
DTXCID00119621	4741-53-1	Tetraphenylphthalic anhydride	0.9	–	–	No	
DTXCID00298329	3711-04-4	[4,4'-Biisobenzofuran]-1,1',3,3'-tetrone	0.9	–	–	No	
DTXCID20298942	1162-64-7	4,7-Diphenyl-2-benzofuran-1,3-dione	0.9	–	–	No	

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DTXCID30413048	73819-76-8	4-Ethynylphthalic Anhydride	0.9	–	–	No	
DTXCID5039274	36978-41-3	[4,5'-Biisobenzofuran]-1,1',3,3'-tetrone	0.9	–	–	No	
DTXCID50511570	954-06-3	4-Phenyl-2-benzofuran-1,3-dione	0.9	–	–	No	
DTXCID601573256	2899-87-8	5-Isobenzofurancarboxylic acid, 1,3-dihydro-1,3-dioxo-, 1,4-butanediyl ester	0.9	–	–	No	
DTXCID10621407	78364-07-5	(4E)-4-(Aminomethylidene)-1H-2-benzopyran-1,3(4H)-dione	–	0.5	–	No	
DTXCID20385624	3453-63-2	3-Methylidene-2-benzofuran-1(3H)-one	–	0.5	–	No	
DTXCID901036998		Unnamed chemical	–	0.5	–	No	
DTXCID50479429	6109-04-2	Dibenzo[d,f][1,2]dioxocine-5,8-dione	–	0.464285702	–	No	
DTXCID401395729	53452-54-3	Oxydi(ethane-2,1-diyl) diprop-2-enoate--2-benzofuran-1,3-dione (1/1)	–	0.463414639	–	No	
DTXCID601183118		Unnamed chemical	–	0.454545468	–	No	
DTXCID201400704	57657-11-1	Oxydi(ethane-2,1-diyl) bis(2-methylprop-2-enoate)--2-benzofuran-1,3-dione (1/1)	–	0.452380955	–	No	
DTXCID20803176	58399-95-4	4-[(Dimethylamino)methylidene]-1H-2-benzopyran-1,3(4H)-dione	–	0.447368413	–	No	
DTXCID50906326		Unnamed chemical	–	0.447368413	–	No	
DTXCID40893938	4767-62-8	(3Z)-3-Ethylidene-1(3H)-isobenzofuranone	–	0.441176474	–	No	
DTXCID501067947		Unnamed chemical	–	0.441176474	–	No	
DTXCID70309916	61658-90-0	NSC508400	–	0.441176474	–	No	
DTXCID80235631	4767-54-8	Isopropylidenephthalide	–	0.441176474	–	No	
DTXCID80246106	28795-86-0	3-(dichloromethylidene)-2-benzofuran-1-one	–	0.441176474	–	No	
DTXCID201244940		Unnamed chemical	–	0.428571433	–	No	
DTXCID60658900	6711-68-8	1H-Furo[3,4-c]pyrrole-1,3(5H)-dione	–	0.428571433	–	No	
DTXCID00537503	46385-43-7	3,3-Dimethyl-3H-2,4,3-benzodioxastannepine-1,5-dione	–	0.419354826	–	No	
DTXCID30248918	6543-57-3	6h,12h,18h,24h-tetrabenzo[b,f,j,n][1,5,9,13]tetraoxacyclohexadecine-6,12,18,24-tetrone	–	0.419354826	–	No	
DTXCID501391370	4664-08-8	Furo[3,4-c]pyridine-1,3-dione	–	0.411764711	–	No	
DTXCID101763808	26647-15-4	4,5-Dihydro-3H-2,6-benzodioxonine-1,7-dione	–	0.40625	–	No	
DTXCID30572487	29246-20-6	3,4,5,6-Tetrahydro-2,7-benzodioxecine-1,8-dione	–	0.40625	–	No	
DTXCID3040566	4743-57-1	Acetic acid, (3-oxo-1(3H)-isobenzofuranylidene)-	–	0.405405402	–	No	
DTXCID90212559		Unnamed chemical	–	0.405405402	–	No	
DTXCID10273889	487-42-3	(3Z)-3-(Phenylimino)-2-benzofuran-1(3H)-one	–	0.400000006	–	No	
DTXCID501066949		Unnamed chemical	–	0.400000006	–	No	
DTXCID50299740	27550-59-0	4-Hydroxyphthalic anhydride	–	0.400000006	–	No	

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DTXCID50501138	17011-53-9	5-Amino-2-benzofuran-1,3-dione	–	0.400000006	–	No	
DTXCID60521428	28418-89-5	5-Iodo-2-benzofuran-1,3-dione	–	0.400000006	–	No	
DTXCID8038437	319-03-9	1,3-Isobenzofurandione, 5-fluoro-	–	0.400000006	–	No	
DTXCID00773883	73198-13-7	3-(3-Oxobutan-2-ylidene)-2-benzofuran-1(3H)-one	–	0.394736856	–	No	
DTXCID00985005		Unnamed chemical	–	0.394736856	–	No	
DTXCID205972	56014-72-3	(E)-3-Propylideneisobenzofuran-1(3H)-one	–	0.394736856	–	No	
DTXCID20929486		Unnamed chemical	–	0.394736856	–	No	
DTXCID30773154	60034-00-6	(3-Oxo-2-benzofuran-1(3H)-ylidene)acetyl chloride	–	0.394736856	–	No	
DTXCID401025532	56014-87-0	1(3H)-Isobenzofuranone, 3-(2-methylpropylidene)-, (3Z)-	–	0.394736856	–	No	
DTXCID40252607	75637-36-4	3-Fluoren-9-ylidene-3H-isobenzofuran-1-one	–	0.394736856	–	No	
DTXCID501063183	88542-70-5	3-(2-Methylpropylidene)-1(3H)-isobenzofuranone	–	0.394736856	–	No	
DTXCID50209400	94704-89-9	(3Z)-3-Propylidene-1(3H)-isobenzofuranone	–	0.394736856	–	No	
DTXCID50652770	40800-81-5	3-(1-Methoxyethylidene)-2-benzofuran-1(3H)-one	–	0.394736856	–	No	
DTXCID701025531	56014-69-8	1(3H)-Isobenzofuranone, 3-(2-methylpropylidene)-, (3E)-	–	0.394736856	–	No	
DTXCID70929855		Unnamed chemical	–	0.394736856	–	No	
DTXCID80822929		Unnamed chemical	–	0.394736856	–	No	
DTXCID90346000	89651-28-5	1(3H)-Isobenzofuranone, 3-[(dimethylamino)methylene]-	–	0.394736856	–	No	
DTXCID207796	17976-43-1	2,4,6,8,3,5,7-Benzotetraoxatriplumbacycloundecin-3,5,7-triylidene, 1,9-dihydro-1,9-dioxo-	–	0.393939406	–	No	
DTXCID60523664	87177-79-5	3,4-Di(9H-fluoren-9-ylidene)oxolane-2,5-dione	–	0.393939406	–	No	
DTXCID20480209	89663-07-0	5-Oxo-5lambda~5~-furo[3,4-c]pyridine-1,3-dione	–	0.388888896	–	No	
DTXCID101702430	60561-31-1	3-(Nitromethylene)-1(3H)-isobenzofuranone	–	0.384615391	–	No	
DTXCID20457971	5693-27-6	1H-2-Benzopyran-1,4(3H)-dione	–	0.382352948	–	No	
DTXCID6035364	16709-50-5	2,9-Benzodioxacyclododecin-1,10-dione, 3,4,5,6,7,8-hexahydro-	–	0.382352948	–	No	
DTXCID20487076	93272-72-1	(3Z)-3-[(2-Methylbutan-2-yl)imino]-2-benzofuran-1(3H)-one	–	0.380952388	–	No	
DTXCID60652867	55937-82-1	3-(1-Methoxypropan-2-ylidene)-2-benzofuran-1(3H)-one	–	0.380952388	–	No	
DTXCID701077994		Unnamed chemical	–	0.380952388	–	No	
DTXCID80985048		Unnamed chemical	–	0.380952388	–	No	
DTXCID401734826	59648-15-6	Furo[3,4-d]pyridazine-5,7-dione	–	0.37931034	–	No	
DTXCID40262423	6007-85-8	1H,3H-Thieno[3,4-c]furan-1,3-dione	–	0.37931034	–	No	
DTXCID10458710	26238-14-2	5-(Trifluoromethyl)-2-benzofuran-1,3-dione	–	0.378378391	–	No	
DTXCID20661314	26038-00-6	1,3-Dioxo-1,3-dihydro-2-benzofuran-5-carbonitrile	–	0.378378391	–	No	
DTXCID701768349	129808-00-0	5,5'-(1,2-Ethynediyl)bis-(1,3-Isobenzofurandione)	–	0.378378391	–	No	

DTXCID	CASRN	Name	Similarity (Cheminform.)	Similarity (GenRA)	AIM	Hazard Data Available in CompTox?	If Yes, Type of Data
DTXCID101661460	339021-18-0	1H-2-Benzopyran-1,3,4-trione 4-(2-phenylhydrazone)	–	0.377777785	–	No	
DTXCID0032867	10416-67-8	1H-Isoindole-1,3(2H)-dione, 2-(trimethylsilyl)-	–	0.375	–	No	
DTXCID701144646		Unnamed chemical	–	0.375	–	No	
DTXCID201077337		Unnamed chemical	–	0.372093022	–	No	
DTXCID40449934	80991-83-9	(3Z)-3-(Butylimino)-2-benzofuran-1(3H)-one	–	0.372093022	–	No	
DTXCID00735921	49543-57-9	4-Hydroxy-4,5-dihydro-3H-2,6-benzodioxonine-1,7-dione	–	0.371428579	–	No	
DTXCID70111511	35512-59-5	2,2-Dimethylpropane-1,3-diyl phthalate	–	0.371428579	–	No	

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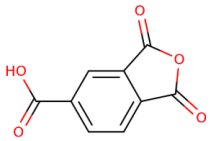
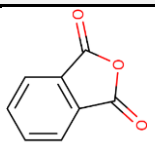
B.2 Physical, Chemical, and Environmental Fate and Transport Similarity

Table_Apx B-2 summarizes physical, chemical, and environmental fate properties of phthalic anhydride and the potential analog, TMA. Phthalic anhydride and TMA are both

- solids at room temperature;
- non-volatile, based on their vapor pressure and Henry's law constants;
- water soluble (6,200 mg/L for phthalic anhydride; 1,036 mg/L for TMA);
- unstable in water and rapidly hydrolyze to *o*-phthalic acid or trimellitic acid, with complete hydrolysis in approximately 2.5 to 7.5 minutes for phthalic anhydride (based on hydrolysis half-life of 30 to 90 seconds, depending on temperature) and approximately 10 minutes for TMA;
- not expected to volatilize from water or soil, given vapor pressure and Henry's law constants;
- primarily partition to ground or surface water; and
- not expected to bioaccumulate.

Overall, EPA preliminarily concludes that given the similarities in physical, chemical, and environmental fate properties, phthalic anhydride and TMA are not expected to behave appreciably different in the environment.

Table_Apx B-2. Summary of Physical, Chemical, and Environmental Fate Properties of Phthalic Anhydride and TMA

Property	TMA[a] (Analog)	Phthalic Anhydride[b]
Structure		
Molecular formula	$C_9H_4O_5$	$C_8H_4O_3$
Molecular weight	192.12 g/mol	148.11 g/mol
Physical form	Solid white flake	Colorless to white lustrous solid
Melting point	165 °C	130.8 °C
Boiling point	390 °C	284.05 °C
Density	1.54 g/mL at 20 °C	1.527 g/cm^3
Vapor pressure	7.6E−05 Pa at 25 °C (OECD 2003) 4E−06 mmHg (PubChem)	5.17E−04 mmHg
Vapor density	6.6 (PubChem)	5.1
Water solubility	1,036 mg/L (assumes no hydrolysis)	6,200 mg/L
Octanol/water partition coefficient (log K_{OW})	1.95 (assumes no hydrolysis or reaction with the alcohol)	1.43
Octanol/air partition coefficient (log K_{OA})	10.231 (KOAWIN v1.10)	5.656
Henry's Law constant	2.40E−09 atm·m^3/mol (HENRYWIN v3.21)	1.70E−08 atm·m^3/mol at 25 °C
Flash point	Not reported	152 °C
Autoflammability	Not reported	584 °C
Viscosity	Not reported	1.125 cP

Property	TMA[a] (Analog)	Phthalic Anhydride[b]
Dissociation constants (pka)	Not reported	NA

[a] Data from (OECD, 2003) or PubChem (https://pubchem.ncbi.nlm.nih.gov/compound/Trimellitic-anhydride, accessed February 2, 2026).
[b] Data from *Draft Physical Chemistry and Fate and Transport Assessment for Phthalic Anhydride* (U.S. EPA, 2026h)

B.3 Toxicological Similarity

This section compares the hazard profiles of phthalic anhydride and the potential analog TMA to determine if the analog is toxicologically similar to the target chemical. Data pertaining to the acute toxicity, skin/eye irritation, skin sensitization, respiratory sensitization and irritation, repeated dose oral toxicity, developmental and reproductive toxicity, genotoxicity, and carcinogenicity of phthalic anhydride and TMA are compared in Table_Apx B-3 and discussed briefly in this section. While data for phthalic anhydride was identified in support of this TSD, data for the potential analog TMA was identified via review of existing assessments by OECD (2003), Australia NICNAS (2014), Health Canada (2019), and ACGIH (2008). Additionally, for the skin and respiratory sensitization endpoints, EPA noted that several studies of phthalic anhydride also evaluated TMA. These comparative studies are also considered in this section.

Acute Toxicity

Both phthalic anhydride and TMA have low acute toxicity via the dermal and inhalation routes of exposure (Table_Apx B-3). Acute dermal LD50 values for phthalic anhydride and TMA are greater than 2,000 mg/kg in rats and/or rabbits, while acute inhalation LC50 values for both phthalic anhydride and TMA are greater than 2,140 mg/m^3 in rats (NICNAS, 2014, 2013; OECD, 2003).

TMA has low acute oral toxicity with an oral LD50 value of 2,730 mg/kg in rats (NICNAS, 2014; OECD, 2003), while phthalic anhydride has an oral LD50 value of 1,530 mg/kg in rats and is classified (GHS) in the EU as Acute Tox. 4 (H302: Harmful if swallowed) (NICNAS, 2013; OECD, 2005).

Eye and Skin Irritation

Both phthalic anhydride and TMA are eye irritants (Table_Apx B-3). Ocular administration of both chemicals in studies of rabbits produced signs of irritation when scored via the Draize method, and for phthalic anhydride, not all effects were reversible during a seven-day observation period (NICNAS, 2014, 2013; OECD, 2005, 2003). Both chemicals are classified (GHS) in the EU as Eye Dam. 1 (H318: Causes serious eye damage).

TMA is considered slightly irritating to skin. When 500 mg/kg TMA was applied via patch to pre-moistened skin of rabbits for four hours, mild irritation was observed (Draize score: 1.7/8.0), but was resolved by the end of the 14-day observation period (NICNAS, 2014; OECD, 2003). In contrast, phthalic anhydride is considered irritating to skin based on the results of OECD TG 404 (Acute Dermal Irritation/Corrosion) studies and human observational studies. Phthalic anhydride, but not TMA, is classified (GHS) as Skin Irrit. 2; H315 (Causes skin Irritation.) in the EU.

Repeated Exposure Oral Toxicity

Both phthalic anhydride and TMA have low systemic toxicity via the oral route of exposure (Table_Apx B-3). As discussed in Section 4.1 of this TSD, no consistent evidence of any specific target organ toxicity was observed across available repeated exposure oral studies of phthalic anhydride. Across

available studies, the only effect consistently observed was decreased body weight gain and/or terminal body weight. For phthalic anhydride, the lowest NOAEL for decreased body weight gain is 375 mg/kg-day from a 2-year dietary study of F344 rats (NCI, 1979).

Similarly, OECD (2003), Australia NICNAS (2014), and Health Canada (2019) have all concluded that TMA has low systemic toxicity via the oral route. Available 90-day dietary studies of rats and beagles support NOAELs of approximately 500 mg/kg-day (highest dose tested, no LOAEL identified). No chronic duration studies of TMA are available.

Developmental and Reproductive Toxicity

As discussed in Section 4.1.3.2 and Section 4.1.3, the weight of scientific evidence indicates that phthalic anhydride is not a developmental toxicant. Similarly, OECD (2003) and Australia NICNAS (2014) have concluded that TMA has "low potential for developmental effects" based on lack of signs of fetal toxicity or teratogenicity in guinea pigs exposed to 0.5 mg/m^3 TMA for six hours/day on GDs 6–15.

No one- or two-generation studies of reproduction are available for phthalic anhydride or TMA.

Carcinogenicity and Genotoxicity

As discussed in Section 6, no evidence of carcinogenicity was observed in male or female rats fed diets containing up to 750 mg/kg-day phthalic anhydride for 2 year, or in male or female mice fed diets containing up to 3,606 to 4,904 mg/kg-day phthalic anhydride for 2 years (NCI, 1979). No 2-year cancer bioassays of TMA have been conducted for any route of exposure.

As discussed in Section 5, phthalic anhydride is negative in the majority of *in vitro* genotoxicity assays. Similarly, OECD (2003) and Australia NICNAS (2014) concluded that the potential for genotoxicity is low for TMA based on results from several *in vitro* assays.

Skin Sensitization

Both phthalic anhydride and TMA are skin sensitizers (Table_Apx B-3). In the EU, both chemicals are classified (GHS) as Skin Sens. 1 (H317: May cause an allergic skin reaction). There is also evidence that phthalic anhydride and TMA have similar potency based on LLNA results (*i.e.,* EC_3 values for phthalic anhydride and TMA range from 0.16–0.36% and 0.2–0.22%, respectively). As discussed previously in Section 4.2.1.1, OECD has developed an AOP for skin sensitization, *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins* (OECD, 2014). Available data for each KE in the adverse outcome is summarized in Table_Apx B-3 and discussed briefly below.

- **KE 1 (Covalent interactions with skin proteins):** Both phthalic anhydride and TMA can covalently bind to proteins. In the DPRA, phthalic anhydride depleted 31 to 75% of synthetic lysine peptide and 1.9 to 16.7% of synthetic cysteine peptide (Bauch et al., 2012; Gerberick et al., 2004), while TMA depleted 44 and 0% of synthetic lysine and cysteine peptides, respectively (Natsch et al., 2013) (Table_Apx B-3). These results indicate that phthalic anhydride and TMA have similar reactivity and both preferentially react with lysine over cysteine.

- **KE 2 (Keratinocyte cellular response):** Both phthalic anhydride and TMA tested negative for sensitization in *in vitro* assays for KE 2 (Table_Apx B-3). Phthalic anhydride was negative in two KeratinoSens assays (Natsch et al., 2013; Bauch et al., 2012) and 1 LuSens assay (Bauch et al., 2012), while TMA was negative in one KeratinoSens assay (Natsch et al., 2013). However, results for both chemicals may be false negatives, as both phthalic anhydride and TMA rapidly hydrolyze to the non-sensitizers *o*-phthalic acid and trimellitic acid, respectively, in the presence of water. Further, and as discussed in OECD TG No. 442D and in Section 4.2.1.1.2, chemicals

that show exclusive reactivity towards lysine-residues may show negative results in both the KeratinoSens and LuSens assays (OECD, 2024).

- **KE 3 (DC response):** Phthalic anhydride showed mixed results in *in vitro* assays for KE 3, while TMA tested negative for sensitization in two *in vitro* assays for KE 3 (Table_Apx B-3). Phthalic anhydride was negative in two h-CLAT assays (Bauch et al., 2012; Nukada et al., 2012) and two of three U-SENS assays (Piroird et al., 2015; Natsch et al., 2013; Bauch et al., 2012), but was positive in one GARDskin assay (NTP, 2026). TMA was negative in one h-CLAT assay (Nukada et al., 2012) and one U-SENS assay (Natsch et al., 2013). As discussed above, these may represent false negatives due to hydrolysis of phthalic anhydride and TMA to the non-sensitizers *o*-phthalic acid and trimellitic acid, respectively, in the presence of aqueous cell culture media.
- **KE 4 (T-cell proliferation):** Both phthalic anhydride and TMA cause T-cell proliferation in the LLNA (Table_Apx B-3). Phthalic anhydride was positive in five LLNAs with EC_3 values ranging from 0.16 to 0.36% (Section 4.2.1.1.4), while TMA was positive in an LLNA with an EC3 value of 0.22% (ECETOC, 2008). These results further indicate that phthalic anhydride and TMA have similar potency for inducing T-cell proliferation.
- **Adverse Outcome (Inflammation upon challenge with allergen):** Both phthalic anhydride and TMA cause the adverse outcome in guinea pigs (Table_Apx B-3). Phthalic anhydride tested positive in 1 GPMT (Basketter and Scholes, 1992) and in one standard and one modified Buehler test (Botham et al., 2005). As described by OECD (2003), TMA was positive in one Buehler test in which guinea pigs were induced with a 30% solution of TMA in DMSO and challenged with a 5% TMA in acetone, and negative in a second modified Buehler assay in which guinea pigs were induced and challenged with 300 mg of TMA powder.

Respiratory Sensitization

Existing assessments by OECD (2005, 2003), Australia NICNAS (2014, 2013), and Health Canada (2019) have all concluded that both phthalic anhydride and TMA are respiratory sensitizers. Further, in the EU both chemicals are classified (GHS) as Resp. Sens. 1 (H344:May cause allergy or asthma symptoms or breathing difficulties if inhaled.) (Table_Apx B-3).

Occupational exposure to both phthalic anhydride and TMA have been linked to increased prevalence of occupational asthma and increased serum antibody levels against phthalic anhydride-HSA and TMA-HSA. Further, *in vivo* experimental animal studies of both phthalic anhydride and TMA have demonstrated similar pulmonary and immune responses, including increased incidence of lung histopathology (*e.g.,* hemorrhagic foci) and increase serum antibodies specific to phthalic anhydride and TMA.

5686 **Table_Apx B-3. Comparison of Phthalic Anhydride and TMA Hazard Profiles**

Endpoint	Phthalic Anhydride (CASRN 85-44-9) [a]	TMA (CASRN 552-30-7) [b]
Acute Oral	LD50 = 1,530 mg/kg (rat) EU Classification (GHS): Acute Tox. 4 (H302: Harmful if swallowed) [c]	LD50 = 2,730 mg/kg (rat)
Acute Inhalation	LC50 >2,140 mg/m^3 (rat)	LC50 > 2,330 mg/m^3 (rat)
Acute Dermal	LD50 > 2,000 mg/kg (rat)	LD50 > 2,000 mg/kg (rabbit); LD50 = 5,600 mg/kg (rat) LD50 >5,600 mg/kg (rats)
Eye Irritation	EU Classification (GHS): Eye Dam. 1 (H318: Causes serious eye damage)[c]	EU Classification (GHS): Eye Dam. 1 (H318: Causes serious eye damage) [d]
Skin Irritation	EU Classification (GHS): Skin Irrit. 2 (H315: Causes skin Irritation)[c]	Slightly irritating (not classified as a skin irritant in the EU)
Repeated Exposure Oral Toxicity	OECD (2005), NICNAS (2013), and Health Canada (2019) have concluded phthalic anhydride has low systemic toxicity via the oral exposure route, with effects generally limited to decreases in body weight (see Section 4.1 for further discussion). Lowest NOAEL for phthalic anhydride = 375 mg/kg-day based on decreased bodyweight gain in 2-year dietary study of F344 rats at LOAEL of 750 mg/kg-day (Section 4.1)	OECD (2003), NICNAS (2014), Health Canada (2019) have concluded TMA has low systemic toxicity via the oral exposure route, with NOAELs of ≈500 mg/kg-day in 13-week dietary studies of rats and beagles (highest dose tested, no LOAEL identified).
Developmental Toxicity	Weight of evidence indicates that phthalic anhydride is not a developmental toxicity (Section 4.1.3.2)	No developmental toxicity observed in guinea pigs or rats exposed to 0.5 mg/m^3 TMA for 6 hrs/day on GDs 6-15 (OECD, 2003) No developmental toxicity study for the oral exposure route.
Reproductive Toxicity	No 1 or 2 generation studies of reproduction available.	No 1 or 2 generation studies of reproduction available.
Genotoxicity	Not genotoxic in majority of *in vitro* assays (Section 5)	Not genotoxic *in vitro* (no *in vivo* studies available)
Carcinogenicity	No evidence of carcinogenicity in male or female rats fed diets containing up to 750 mg/kg-day phthalic anhydride for 2-years (Section 6). No evidence of carcinogenicity in male or female mice fed diets containing up to 3,606 to 4,904 mg/kg-day phthalic anhydride for 2-years (Section 6).	No 2-year cancer bioassays available.
Skin Sensitization	EU Classification (GHS): Skin Sens. 1 (H317: May cause an allergic skin reaction) [c] **OECD Skin Sensitization AOP (OECD, 2014)** KE 1 (Covalent Binding to Proteins) -DPRA: lysine depletion (75%), cysteine depletion (1.9%) (Gerberick et al., 2004)	EU Classification (GHS): Skin Sens. 1 (H317: May cause an allergic skin reaction) [d] **OECD Skin Sensitization AOP (OECD, 2014)** KE 1 (Covalent Binding to Proteins) - DPRA: Lysine depletion (44%), Cysteine depletion (0%) (Natsch et al., 2013)

Endpoint	Phthalic Anhydride (CASRN 85-44-9) [a]	TMA (CASRN 552-30-7) [b]
	KE 2 (Events in Keratinocytes) -KeratinoSens: Negative in 2 assays (Natsch et al., 2013; Bauch et al., 2012) -LuSens: Negative (Bauch et al., 2012) KE 3 (Events in DCs) -h-CLAT: Negative in 2 assays (Bauch et al., 2012; Nukada et al., 2012) -U-SENS: Negative in 2 of 3 assays (Piroird et al., 2015; Natsch et al., 2013; Bauch et al., 2012) -$GARD_{Skin}$: Positive (NTP, 2026) KE 4 (T-cell Proliferation) - LLNA: Positive in 5 assays [EC3 = 0.16 - 0.36%] (Section 4.2.1.1.4) Adverse Outcome (Inflammation Upon Challenge with Allergen) - GPMT: Positive in 1 assay (Basketter and Scholes, 1992) - Buehler Test: Positive in 1 assay (Botham et al., 2005)	KE 2 (Events in Keratinocytes) - KeratinoSens: Negative (Natsch et al., 2013) KE 3 (Events in DCs) - h-CLAT: Negative (Nukada et al., 2012) - U-SENS: Negative (Natsch et al., 2013) KE 4 (T-cell Proliferation) - LLNA: Positive [EC3 = 0.22%] (ECETOC, 2008) Adverse Outcome (Inflammation Upon Challenge with Allergen) - Buehler Test: Positive in 1 of 2 assays (OECD, 2003)
Respiratory Sensitization	Classification (GHS): Resp. Sens. 1 (H344:May cause allergy or asthma symptoms or breathing difficulties if inhaled.) [c]	Classification (GHS): Resp. Sens. 1 (H344: May cause allergy or asthma symptoms or breathing difficulties if inhaled.) [d]
Respiratory Irritation	Classification (GHS): STOT SE 3 (H335: May cause respiratory irritation.) [c]	Classification (GHS): STOT SE 3 (H335: May cause respiratory irritation.) [d]

AOP = adverse outcome pathway; DPRA = direct peptide reactivity assay; EU = European Union; LOAEL = Lowest-observed-adverse-effect level; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NOAEL = No-observed-adverse-effect level; OECD = Organisation for Economic Co-operation and Development

[a] Data from assessments by OECD (2005), Australia NICNAS (2013) and Health Canada (2019), unless otherwise noted.

[b] Data from assessments by OECD (2003), Australia NICNAS (2014) and Health Canada (2019), unless otherwise noted.

[c] Phthalic anhydride GHS classification available at: https://chem.echa.europa.eu/100.001.461/overview (accessed February 2, 2026)

[d] TMA GHS classification available at: https://chem.echa.europa.eu/100.008.190/overview (accessed February 2, 2026)

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B.4 Summary of Available Laboratory Animal Inhalation Studies of TMA

EPA reviewed existing assessments of TMA to identify reasonably available experimental animal inhalation studies of TMA, including assessments by OECD (2003), Australia NICNAS (2014), Health Canada (2019), and ACGIH (2008). EPA identified several acute and intermediate duration studies of TMA (all conducted with SD rats) ranging in duration from 1 to 14 days (Zeiss et al., 1989; Zeiss et al., 1988; Leach et al., 1987; Zeiss et al., 1987), as well as one 13-week inhalation study of SD rats, which include a 6.5-week interim sacrifice (Leach et al., 1989; IIT Research Institute, 1988). Reasonably available inhalation studies reviewed by EPA are discussed briefly below and are summarized in Table_Apx B-4.

Respiratory effects consistent with sensitization and lung damage were observed consistently across available intermediate and subchronic duration studies of TMA. In male SD rats exposed to 0 or 100 µg/m^3 TMA for 6 hours/day for 2, 6, and 10 days, evidence of lung damage (*e.g.,* increased incidence of hemorrhagic foci, relative lung weight and lung volume) and antibody responses (*e.g.,* increased serum and/or BALF levels of total antibody, IgG, IgA, IgM antibody) were apparent by day 6, with the magnitude of the response increasing by day 10 (Zeiss et al., 1988). Similar results were obtained in a series of three studies conducted by Zeiss et al. (1989). In the first study, male SD rats were exposed to 0 or 500 µg/m^3 TMA for 6 hours on study days 1 and 5, and then challenged with a single 6-hour exposure to 500 µg/m^3 TMA on study day 29. Serum levels of IgG, IgM, and IgA antibodies against TMA-RSA (TMA-rat serum albumin conjugate), relative lung weight, and incidence of hemorrhagic foci were increased in rats sacrificed after the challenge on study day 30 (Zeiss et al., 1989). In a second study of similar design, male SD rats were exposed to 0 or 330 µg/m^3 TMA for 6 hours on study days 1, 5, and 10, and then challenged with a single 6-hour exposure to 300 µg/m^3 TMA on study day 22. Similar to the first study, serum levels of IgG, IgM, and IgA antibodies against TMA-RSA, relative lung weight and volume, and incidence of hemorrhagic foci were increased, with the magnitude of the response being greater in challenged rats compared to the non-challenged rats (Zeiss et al., 1989). Finally, in a third study designed to evaluate the time-course of the antibody response, male SD rats were exposed to 0 or 500 µg/m^3 TMA for 6 hours on study days 1, 5, and 10, and then challenged with a single 6-hour exposure to 540 µg/m^3 TMA on study day 29. Serum levels of IgM, IgA, and IgG antibodies specific to TMA-RSA increased starting between study days 5 to 7 and peaked on study day 20. Similar to the first two studies, increase in incidence of hemorrhagic foci was also observed in rats on study day 30 (Zeiss et al., 1989).

Similar results were obtained in a dose-response study in which male and female SD rats were exposed to 0, 10, 30, 100, and 300 µg/m^3 TMA for 6 hours/day, 5 days/week for 2 weeks and then sacrificed 18-hours post-exposure (Group 1), 12-days post exposure (Group 2), 12-days post exposure with a single 6-hour TMA challenge to the same concentration of TMA (Group 3), or 12-weeks post-exposure (Group 4) (Leach et al., 1987; Zeiss et al., 1987). In rats sacrificed immediately post-exposure (Group 1), dose-related increases in relative lung weight (≥30 µg/m^3), gross hemorrhagic foci (≥100 µg/m^3), alveolar macrophage accumulation and alveolar hemorrhage (≥30 µg/m^3), lung pneumonitis (300 µg/m^3), non-specific IgG levels in alveolar macrophages and serum antibody against TMA-RSA conjugate (≥10 µg/m^3) were observed, supporting a LOAEC of 10 µg/m^3 (no NOAEC identified). Most of these effects were found to be reversible after a 12-day recovery period (Group 2), while all effects were resolved after a 12-week recovery (Group 4). However, effects on the lung and antibodies recurred in rats rested for 12-days and then challenged with a single 6-hour exposure to TMA (Group 4), with effects observed in the lowest exposure group (*i.e.,* a dose-related increase in IgG levels in alveolar macrophages at ≥10 µg/m^3).

In a subchronic duration dose-response study, male and female SD rats were exposed to 0, 2, 15, and 50 µg/m^3 TMA for 6 hours/day, 5 days/week for 13 weeks (Leach et al., 1989; IIT Research Institute, 1988). The study included a 6.5-week interim sacrifice of male rats as well as 3- and 38-week recovery groups (males only), which included a single 6-hour challenge to 50–62 µg/m^3 TMA prior to sacrifice. In males at interim-sacrifice, dose-related increases in relative lung weight and volume (≥15 µg/m^3), incidence of gross external hemorrhagic lung foci (50 µg/m^3), histologic incidence of multifocal lobular bronchopneumonia and focal hemorrhage (≥2 µg/m^3), and serum levels of antibodies against TMA-RSA conjugate (≥2 µg/m^3) were observed, supporting a LOAEC of 2 µg/m^3 (no NOAEC identified). After 13-weeks of exposure to TMA, some evidence of immunologic tolerance was observed, as demonstrated by a reduced response for most effects. Relative lung weight and volume was generally increased in male and female rats; however, the effect did not reach statistical significance. Similarly, incidence of gross external hemorrhagic lung foci was increased in male, but not female rats, at 50 µg/m^3 TMA, while histologic incidence (only evaluated in control and high-dose group) of multifocal lobular bronchopneumonia was increased in all male and female rats at 50 µg/m^3 TMA, while only a slight increase in histologic incidence of hemorrhage was observed in male (incidence: 0 [control], 30% [50 µg/m^3]) and female rats (incidence: 0 [control], 10% [50 µg/m^3]). Serum levels of antibodies against TMA-RSA conjugate were significantly increased in both male and female rats at 2 µg/m^3 TMA and above, however, the magnitude of the antibody response was lower in males at 13 weeks compared to 6.5 weeks (*e.g.,* serum antibody levels were ≈4-fold higher at 6.5 weeks). In the 3-week recovery group, effects on relative lung weight and volume and serum antibodies against TMA-RSA persisted in both non-challenged and rats challenged with a TMA inhalation exposure, however, incidence of hemorrhagic lung foci was no longer significant in non-challenged or challenged rats. In the 38-week recovery group, no significant effects on relative lung weight or volume, incidence of hemorrhagic lung foci, or serum antibody levels were observed in non-challenged rats, while a slight increase in relative lung weight was observed in challenged rats.

5761 **Table_Apx B-4. Summary of Inhalation Studies of TMA**

Brief Study Description	NOAEC/ LOAEC (µg/m³)	Effect at LOAEC	Effects at Higher Doses
Intermediate duration (>1–30 days)			
Male SD rats (15/group/timepoint) exposed to 0 (filtered air) or 100 µg/m³ micronized TMA powder for 6 hours/day for 2, 6, or 10 days (Zeiss et al., 1988)	None/ 100	↑ incidence of hemorrhagic lung foci; ↑ total IgG, IgA, IgM antibody in serum and BALF after 6–10 days of exposure	Effects after 2 days of exposure - No effect on lung foci #, relative lung weight, lung displacement volume, serum or BALF total or TMA-specific antibodies Effects after 6–10 days of exposure - ↑ incidence of hemorrhagic lung foci, ↑ relative lung weight, ↑ lung displacement volume after 6 and 10 days (magnitude of effect larger after 10 days for all outcomes) - ↑ total antibody, IgG, IgA, IgM antibody in serum and BALF after 6 and 10 days (magnitude of effect larger after 10 days) Limitations: - Limited exposure characterization details provided (*e.g.,* measured air concentration, MMAD, GSD, exposure apparatus not reported)
Male SD rats (5/group) exposed (whole body) to target concentrations of 0 (filtered air only), 10, 30, 100, 300 µg/m³ TMA for 6 h/day for 5 days and then sacrificed. Measured concentrations: 0, 11.1, 37.6, 102.6, 258.4 µg/m³ TMA (MMAD = 1.0–1.16 µm) (Leach et al., 1987; Zeiss et al., 1987)	300/ None	None	Unaffected outcomes: - Survival, incidence of clinical signs, body weight gain, clinical chemistry, hematology, bone marrow cellularity, organ weight (including lung), gross or microscopic appearance of any organ (including lung), serum antibody levels Limitations: -GSD not reported or calculatable; authors report that 96% of particles were smaller than 10 µm.
Male and female SD rats (35 males and 20 females/group) exposed to target concentrations of 0 (filtered air only), 10, 30, 100, 300 µg/m³ TMA for 6 h/day, 5 days/wk for 2 weeks. Measured concentrations: 0, 11.1, 37.6, 102.6, 258.4 µg/m³ TMA (MMAD 1.0–1.16 µm). Exposed rats split into 4 groups. Group 1 sacrificed 18-hours after the final exposure (10/sex/group) Group 2 sacrificed 12 days post-exposure (10 males and 5 females per group);	None/ 10	↑ IgG levels in alveolar macrophages & serum antibody against TMA-RSA conjugate	Group 1 Results (no recovery) - ↑ relative lung weight (≥30 µg/m³) - ↑ # of gross hemorrhagic foci (≥100 µg/m³) - ↑ # of alveolar macrophage accumulation & alveolar hemorrhage values (≥30 µg/m³) - ↑ severity of lung pneumonitis (300 µg/m³) - ↑ C3 levels in alveolar macrophages (≥30 µg/m³) - ↑ non-specific IgG levels in alveolar macrophages (≥10 µg/m³) - ↑ serum antibody against TMA-RSA conjugate (≥10 µg/m³) Group 2 Results (12-day recovery) - ↑ relative lung weight (300 µg/m³) - ↑ # of gross hemorrhagic foci (100 µg/m³) (no dose-response)

Brief Study Description	NOAEC/ LOAEC (µg/m^3)	Effect at LOAEC	Effects at Higher Doses
Group 3 sacrificed 12 days post-exposure with 6-hour challenge to same concentration of TMA as originally exposed (10 males and 5 females per group) Group 4 sacrificed 12 weeks post-exposure (5 males/group) (Leach et al., 1987; Zeiss et al., 1987)			- ↑ # of alveolar macrophage accumulation (300 µg/m^3) Group 3 Results (12-day recovery with TMA challenge) - ↑ relative lung weight (≥100 µg/m^3) - ↑ # of gross hemorrhagic foci (≥100 µg/m^3) - ↑ # of alveolar macrophage accumulation (≥30 µg/m^3) & alveolar hemorrhage values (300 µg/m^3) - ↑ severity of lung pneumonitis (100 µg/m^3) (no dose-response) - ↑ C3 levels in alveolar macrophages (≥100 µg/m^3) - ↑ non-specific IgG levels in alveolar macrophages (≥10 µg/m^3) Group 4 Results (12-week recovery) - Unaffected outcomes: relative lung weight, incidence of gross hemorrhagic lung foci, alveolar macrophage accumulation, alveolar hemorrhage value, C3 levels in alveolar macrophages, alveolar macrophage IgG levels Unaffected outcomes (all treatment groups): - Survival, incidence of clinical signs, body weight gain, clinical chemistry, hematology, bone marrow cellularity, organ weight (other than lung), gross or microscopic appearance of any organ (other than lung)
Male SD rats (6/dose) exposed to a target concentration of 500 µg/m^3 TMA for 6 h/day on study days 1, 5, and 10. Blood samples were collected every second day on study days 1–26. On study day 29, rats were challenged with a 6-hour exposure to 540 µg/m^3 TMA and 18 hours later, rats were sacrificed (Zeiss et al., 1989)	None/ 500	↑ serum IgG, IgM , IgA antibody to TMA-RSA conjugate & ↑ incidence of lung hemorrhagic foci	- ↑ serum IgM and IgA antibody to TMA-RSA starting on day 5 and peaking on day 20 - ↑ serum IgG antibody to TMA-RSA increased starting on study day 7 and peaking at day 20, with a subsequent plateau (IgG antibody response was at least 10 times higher than IgA or IgM response) - ↑ incidence of lung hemorrhagic foci
Male SD rats (18/dose) exposed to a target concentration of 330 µg/m^3 TMA for 6 h/day on study days 1, 5, and 10. Rats were allowed to rest for 12 days, and on study day 22, 12 of the 18 animals were challenged with a 6-hour exposure to 300 µg/m^3 TMA. 18 hours later on study day 23 all rats were sacrificed (Zeiss et al., 1989)	None/ 330	↑ serum IgG, IgM , IgA antibody to TMA-RSA conjugate & ↑ incidence of lung hemorrhagic foci	- ↑ serum IgG, IgM, and IgA antibody to TMA-RSA - ↑ incidence of lung hemorrhagic foci (reduced response in non-challenged animals) - ↑ relative lung weight and volume (reduced response in non-challenged animals) - Serum IgG, IgM, IgA correlated with increased relative lung weight and hemorrhagic foci in challenged rats
Male SD rats (8/dose) exposed to a target concentration of 500 µg/m^3 TMA for 6 h/day on study days 1 and 5. Animals were then allowed to rest for 24	None/ 500	↑ serum IgG, IgM , IgA antibody to TMA-RSA	- ↑ serum IgG, IgM, and IgA antibody to TMA-RSA - ↑ incidence of lung hemorrhagic foci & relative lung weight

Brief Study Description	NOAEC/ LOAEC (µg/m^3)	Effect at LOAEC	Effects at Higher Doses
days, and on study day 29 animals were challenged with a 6-hour exposure to 500 µg/m^3 TMA. 18 hours later on study day 30 animals were sacrificed (Zeiss et al., 1989)		conjugate & ↑ incidence of lung hemorrhagic foci	- Serum IgG, IgM, IgA correlated with increased relative lung weight and hemorrhagic foci in challenged rats
Subchronic duration (>30–90 days)			
Male SD rats (10/dose) exposed to target concentrations of 0, 2, 15, 50 µg/m^3 TMA (measured: 2.2, 15.4, 53.5 µg/m^3) for 6 hours/day, 5 days/week for 6.5 weeks (MMAD ± GSD: 1.7 µm ± 1.4; 2.2 µm ± 1.4; 2.2 µm ± 1.4) (Leach et al., 1989; IIT Research Institute, 1988)	None/ 2	↑ TMA-specific serum antibody & incidence of multifocal lobular bronchopneumonia	- ↑ relative lung weight and volume (≥15 µg/m^3) - ↑ incidence of gross external hemorrhagic lung foci (5 ± 5, 45 ± 69, 77 ± 65, 156 ± 98, statistically significant only at 50 µg/m^3) - ↑ histologic incidence of multifocal lobular bronchopneumonia (≥2 µg/m^3) (incidence: 0%, 90%, 100%, 100%; severity score: 0, 1.6, 2.3, 2.9 out of possible 4.0) & focal hemorrhage (incidence: 0%, 30%, 40%, 60%) - ↑ TMA-specific serum antibody (≥2 µg/m^3) Unaffected Outcomes - Survival, clinical signs, body weight, absolute or relative organ weight (other than lung), hematology and differential white blood cell counts, gross necropsy results (other than external hemorrhagic lung foci), histopathology in tissues other than lung
Male and female SD rats exposed to target concentrations of 0, 2, 15, 50 µg/m^3 TMA (measured: 2.2, 15.4, 53.5 µg/m$^{3)}$ for 6 hours/day, 5 days/week for 13 weeks (MMAD ± GSD: 1.7 µm ± 1.4; 2.2 µm ± 1.4; 2.2 µm ± 1.4). Animals were euthanized immediately (no recovery; 10/sex/dose); after a 3-week recovery (12 males/group) in which 6 rats from each group were challenged with a single 6-hour exposure to 50 µg/m^3 TMA and 6 from each group remained unchallenged; and after a 38-week recovery (6 males/group for all groups except high-dose, which included 12 males), in which 6 high-dose males were challenged with a single 6-hour exposure to 62 µg/m^3 TMA and all other rats remained unchallenged (Leach et al., 1989; IIT Research Institute, 1988)	None/ 2	↑ TMA-specific serum antibody	No Recovery Group (both sexes) - ↑ relative lung weight and volume (both sexes, not statistically significant) -↑ incidence of gross external hemorrhagic lung foci in male, but not female, rats (50 µg/m^3) - ↑ histologic incidence of multifocal lobular bronchopneumonia (only evaluated in 0 and 50 µg/m^3 groups) (incidence: 0%, 100% in both sexes) & hemorrhage (incidence: 0, 30% [males]; 0, 10% [females]) - ↑ TMA-specific serum antibody (≥2 µg/m^3) (both sexes) - Unaffected: Survival, clinical signs, body weight, absolute or relative organ weight , hematology and differential white blood cell counts, clinical chemistry, gross necropsy results (other than hemorrhagic lung foci), histopathology in tissues other than lung 3-week recovery group (males only) - ↑ relative lung weight (≥15 µg/m^3 in both challenged and unchallenged groups) - ↑ relative lung volume (≥15 µg/m^3 in challenged group only)

Brief Study Description	NOAEC/ LOAEC (µg/m^3)	Effect at LOAEC	Effects at Higher Doses
			- ↑ histologic incidence of multifocal lobular bronchopneumonia (50 µg/m^3 in both challenged and unchallenged groups) - ↑ TMA-specific serum antibody (≥15 µg/m^3 in both challenged and unchallenged groups) - Unaffected: survival, clinical signs, body weight, lung volume, incidence of gross lesions (including gross external lung foci), organ weights (other than lung), histopathology 38 week recovery group (males only) - ↑ relative lung weight (challenge group only) - ↑ TMA-specific serum antibodies in all dose groups (effect not statistically significant) - Unaffected: survival, clinical signs, body weight, organ weight (including lung), relative lung volume, gross pathology, histopathology
Male SD rats (6/dose) exposed to target concentration of 50 µg/m^3 TMA (measured: 53.5 µg/m^3) for 6 hours/day, 5 days/week for 13 weeks (MMAD ± GSD: 2.2 µm ± 1.4) and then held for 32 weeks without further exposure. Serum TMA-specific antibody levels determined at weekly to bi-weekly intervals (Leach et al., 1989; IIT Research Institute, 1988)	None/50	↑ TMA-specific serum antibody	- ↑ TMA-specific serum antibody levels peaked after 6 weeks of exposure, then declined to a moderate level during the rest of the exposure, increased after the exposure ended, and then declined again starting around week 19 to a low, but significant level during the entirety of the recovery period Unaffected Outcomes - Survival, clinical signs
Male SD rats (14/dose) exposed to target concentration of 50 µg/m^3 TMA (measured: 53.5 µg/m^3) for 6 hours/day, 5 days/week for 10 weeks (MMAD ± GSD: 2.2 µm ± 1.4). Two rats were euthanized and necropsied after 1, 2, 3, 4, 6, 8, and 10 weeks of exposure (Leach et al., 1989; IIT Research Institute, 1988)	None/50	↑ incidence of lung hemorrhagic foci	- Incidence of hemorrhagic lung foci peaked after 2 weeks (≈350 foci) of exposure, then declined through week 6 and remained relatively constant through week 10 (≈100 foci) Unaffected Outcomes - Survival, clinical signs

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B.5 Summary of Available Human Evidence for TMA

EPA reviewed epidemiological evidence and conclusions from previous assessments of TMA conducted by ACGIH (2008), OECD (2003), and Health Canada (2019). Health Canada's assessment included a literature search from 2001 to October 2016, and did not identify new epidemiological studies that would lead to a different conclusion than that of OECD. Therefore, EPA reviewed the studies evaluated by OECD and ACGIH as described below. The ACGIH and OECD SIDS assessments identified TMA as a respiratory sensitizer.

Twenty-four occupational exposure studies were reviewed by EPA,[7] 6 of which were case studies or case series. All studies focused on TMA or other acid anhydride exposure in worker populations. Common observations across studies included increased antibodies specific to TMA, asthma, allergic rhinitis, and late respiratory systemic syndrome (LRSS), consistent with respiratory sensitization. There is evidence that TMA exposure can also result in upper respiratory irritation, reflected by symptoms such as sneezing, rhinitis, and watery eyes. However, there is little to no antibody activity and the irritancy effect does not require a latent period after initial exposure (Letz et al., 1987; Zeiss et al., 1977). In comparison, the latent period for sensitized workers that precedes symptoms of asthma or rhinitis lasts weeks or years (Bernstein et al., 1982; Zeiss et al., 1977).

Similar to phthalic anhydride, the respiratory and immune mediated symptoms experienced by workers are mediated by IgG and IgE antibodies, and the presence of TMA-specific antibodies indicates exposure. Inhaled TMA forms antigenic complexes (*e.g.,* TMA-HSA complexes) and specific antibodies to those complexes. Topping et al. (1986) demonstrated that the IgE response to TMA following inhalation exposure is primarily due to the hapten complex of TMA (*e.g.,* TMA-HSA complexes). Grammer et al. (1998) investigated the clinical and immunological effects of TMA exposure, following 181 participants for up to five years. The study found that individuals with IgE antibodies against TMA-HSA were more likely to develop asthma, with symptoms appearing in some after 1 year and more after five years. Similar patterns were observed for IgG antibodies, reinforcing the predictive value of these antibodies for respiratory disease development. Indeed, Sale et al. (1981) reported that total antibody level and IgG antibody activity against TMA-HSA could discriminate between workers with LRSS and asymptomatic workers. Letz et al. (1987) conducted a cross-sectional study measuring respiratory symptoms, lung function, and serum antibody levels to TMA in workers at a barrel manufacturing facility. Four employees exhibited symptoms consistent with TMA-induced irritant effects, with some showing LRSS-like symptoms and increased levels of IgG against TMA-HSA. Two workers experienced significant decreases in peak expiratory flow rates after shifts, highlighting the acute impact of exposure. Gerhardsson et al. (1992) compared IgG subclass antibodies against TMA-HSA in workers without lung illness to subgroups of workers with lung disease, such as those with LRSS or asthma/rhinitis, to workers without lung illness. No significant differences were found between the groups, despite higher total antibody levels in TMA-exposed workers, suggesting that IgG subclass levels may not directly correlate with disease presence.

Additional studies have reported various respiratory and immune mediated conditions following workplace exposure to TMA. Patterson et al. (1979) evaluated pulmonary disease symptoms in workers exposed to TMA fumes, comparing them to laboratory workers. Pulmonary function and disease

[7] Studies of human exposure to TMA reviewed by EPA include (Grammer et al., 2002; Grammer et al., 2000; Grammer et al., 1999; Barker et al., 1998; Grammer et al., 1998; Piirilä et al., 1997; Gerhardsson et al., 1993; Grammer et al., 1993; Gerhardsson et al., 1992; Grammer et al., 1992; Boxer et al., 1987; Letz et al., 1987; Rosenman et al., 1987; Topping et al., 1986; McGrath et al., 1984; Bernstein et al., 1983; Bernstein et al., 1982; Rivera et al., 1981; Sale et al., 1981; Ahmad et al., 1979; Patterson et al., 1979; Fawcett et al., 1977; Rice et al., 1977; Zeiss et al., 1977)

symptoms were evaluated via clinical survey, and IgE antibody activity against TMA-HSA as well as titers against TMA erythrocytes were measured. Workers with TMA-induced pulmonary disease-anemia syndrome had antibody concentrations similar to those with other immunologic respiratory diseases. Bernstein et al. (1982) evaluated clinical and immunological effects in 20 workers exposed to TMA via inhalation using a questionnaire and measurements of serum antibody levels of TMA-HSA (IgE and IgG). Exposure was not quantified; workers were grouped as "high" or "low" exposure groups based on job title, and there was co-exposure with phthalic anhydride. Outcomes were evaluated by a local physician with experience with TMA-related respiratory disease The questionnaire identified nonirritant, immunologic symptoms caused by TMA primarily in the high-exposure group. Specific IgG, IgE, and total antibody binding to TMA-HSA were found exclusively in the high-exposure workers.

Zeiss et al. (1977) found that chemical plant workers with late-onset asthma with systemic symptoms had higher IgG antibody levels against TMA-HSA. One employee with IgE-mediated asthma and two employees with late-onset asthma had rheumatoid factor in high titer. Three employees who were representative of the three clinical conditions showed lymphocyte reactivity of TMA-HSA. One worker with high levels of IgE antibody specific for TMA-HSA and severe symptoms of asthma and acute rhinitis showed leukocyte histamine release to TMA-HSA. A later study by Zeiss et al. (1990) reported immunologic lung diseases in a population of 196 workers involved in the manufacture of TMA. A subset of those workers (17) had IgE-mediated asthma/rhinitis with a positive prick test to TM-HSA and IgE antibody.

A few studies provided information that demonstrate an exposure gradient. One report of 46 employees exposed to TMA by Grammer et al. (1992) found that workers with low level exposure to TMA have a corresponding low incidence of immunologically mediated disease due to TMA. An occupational cohort study by the same authors (Grammer et al., 1999) evaluated the incidence of immunologically mediated respiratory disease in 286 workers employed at a facility that manufactures TMA for 3 years. Workers were assigned one of five exposure class groupings based on personal air monitoring for TMA, with class 1 being the highest exposure and class 5 being the lowest. Of the 28 individuals in exposure class 1, 8 (29%) developed disease; of the 57 class 2 employees, 2 (4%) developed disease; of the 79 class 3 employees, 4 (5%) developed disease. Of the 98 class 4 employees and the 24 class 5 employees, none developed disease Another analysis of factory workers exposed to multiple acid anhydrides by Barker et al. (1998) did not initially find an exposure-response relationship. However, when exposure was limited to a factory that used only TMA, a higher prevalence of acid anhydride sensitization and increased work-related respiratory symptoms was observed with full shift exposure. This relationship was evident within the 40 mg/m^3 current occupational exposure guideline and was not substantially altered by atopy or smoking. Barker et al. (1998) also reported that individuals exposed to TMA suffered from anemia, gastrointestinal hemorrhage, and respiratory failure; intra-alveolar bleeding and injury to alveolar lining cells were observed in lung biopsy tissue.

Four studies provided evidence on reversibility of some effects following removal of exposure, where introduction of workplace controls or other means to reduce exposure led to improved health outcomes. Grammer et al. (2000) reported improved outcomes in workers with immunologic lung illnesses after transferring to low-exposure jobs, noting improvements in symptoms, pulmonary function, and antibody levels. Individuals with improved symptoms had lower IgE against TMA-HSA. McGrath et al. (1984) evaluated two groups of workers at risk of developing TMA-induced lung disease before and after workplace control modifications. Observations included allergic rhinitis, asthma, and late respiratory systemic syndrome among workers, with some showing antibodies against TMA-HSA(Boxer et al., 1987) documented the impact of stricter environmental control measures on TMA exposure, noting a decline in antibody levels and clinical symptoms among workers. Airborne TMA concentrations were

measured at job stations and outcomes were determined by physical exam from a physician. McGrath et al. (1984) supported these findings; following the implementation of various workplace control measures none of the employees in the study experienced substantial total or specific IgE antibody binding to TMA-HSA or TMA-induced immunologic illness.

Bernstein et al. (1983) examined immunologic and clinical effects in workers exposed to TMA. Initial evaluations showed five of 12 workers had antibodies against TMA-HSA, with three exhibiting LRSS. Increased IgE binding was noted in a maintenance operator with rhinitis, while an asymptomatic extruder operator showed increased total antibody and TMA-specific IgE. Improved ventilation in the facility reduced airborne dust concentrations, correlating with a decline in antibody levels and symptoms.

Case Reports and Case Series

In a case series, Grammer et al. (2002) evaluated 25 individuals with TMA-induced asthma. The authors concluded that rhinitis and conjunctivitis symptoms were frequent in individuals with occupational asthma because of TMA, and that these effects frequently appear before respiratory symptoms. Additional case-reports of workers exposed to TMA and/or other acid anhydrides report the development of various syndromes including allergic alveolitis (Piirilä et al., 1997), chemical pneumonitis (Rice et al., 1977), immediate asthmatic reactions (Fawcett et al., 1977), pulmonary hemorrhage and hemolytic anemia (Ahmad et al., 1979) and other pulmonary and hematologic damage (Rivera et al., 1981). In some studies, the clinical observations and lung functional deficits corresponded to increases in antibodies against TMA-HSA, illustrating the link between TMA exposure and severe respiratory and hematologic conditions (Rivera et al., 1981; Ahmad et al., 1979)

Mechanistic Considerations

Similar to phthalic anhydride, cross-reactivity has been described for IgE and IgG antibodies against TMA-HSA, likely due to shared antigenic determinants among different antigens (*i.e.,* phthalic anhydride and TMA are structurally similar) (Lowenthal et al., 1994; Gerhardsson et al., 1993; Rosenman et al., 1987; Zeiss et al., 1977). For instance, in Gerhardsson et al. (1993), IgG antibodies against TMA-HSA bind to TMA-HSA with the strongest affinity, but also bind to hapten conjugates of other acid anhydrides such as phthalic anhydride, demonstrating evidence of cross-reactivity with other phthalates.

B.6 Weight of Scientific Evidence Conclusions: TMA

EPA has reached the preliminary conclusion that TMA is a suitable analog for phthalic anhydride. *Overall, EPA has robust confidence that TMA is a suitable analog based on the following weight of scientific evidence considerations:*

- Phthalic anhydride and TMA are structurally similar, with a Tanimoto coefficient of 1.0, as identified in the Cheminformatics Module following EPA's search for candidate analogs based on structural similarity (Appendix B.1).
- Phthalic anhydride and TMA share similar physical, chemical, and environmental fate properties. Overall, phthalic anhydride and TMA are not expected to behave appreciably different in the environment (Appendix B.2).
- Reasonably available animal toxicology studies support the conclusions that TMA is toxicologically similar to phthalic anhydride (Appendix B.3).

- Both TMA and phthalic anhydride also have low systemic toxicity via the oral route of exposure (Appendix B.3). OECD (2005, 2003), Australia NICNAS (2014, 2013), and Health Canada (2019) have reached the same conclusion.
- Reasonably available *in vivo* animal studies, *in vitro* mechanistic studies, and human epidemiologic studies, indicate that both phthalic anhydride and TMA are dermal sensitizers. Further, LLNA EC3 values for phthalic anhydride and TMA are 0.16% and 0.22%, indicating similar potency (Appendix B.3).
- Reasonably available *in vivo* animal studies and human epidemiologic studies, indicate that both phthalic anhydride and TMA are respiratory sensitizers (Appendices B.3, B.4, B.5).
- Repeated-dose inhalation studies of TMA are available. EPA identified several reasonably available inhalation dose-response studies of TMA, including 2-week, 6.5-week, and 13-week inhalation studies of male and female SD rats (Leach et al., 1989; IIT Research Institute, 1988) (Appendix B.4).

OECD (2005, 2003), Australia NICNAS (2014, 2013), Health Canada (2019), and ACGIH (2025, 2008) have also concluded that both phthalic anhydride and TMA are both dermal and respiratory sensitizers. Further, phthalic anhydride and TMA are both classified (GHS) as Skin Sens. 1 (H317: May cause an allergic skin reaction) and Resp. Sens. 1 (H344: May cause allergy or asthma symptoms or breathing difficulties if inhaled.) in the EU.

Appendix C CALCULATING DAILY ORAL HUMAN EQUIVALENT DOSES AND HUMAN EQUIVALENT CONCENTRATIONS

All data considered for oral PODs are obtained from oral animal toxicity studies in rats or mice following exposures to phthalic anhydride or *o*-phthalic acid. Because toxicity values for phthalic anhydride and *o*-phthalic acid are from oral animal studies, EPA must use an extrapolation method to estimate human equivalent doses (HEDs). The preferred method would be to use chemical-specific information for such an extrapolation. EPA did not identify any reasonably available physiologically based pharmacokinetic (PBPK) models to extrapolate HEDs between species for phthalic anhydride or *o*-phthalic acid. EPA therefore relied on the guidance from U.S. EPA (2011b), which recommends scaling allometrically across species using the three-quarter power of body weight ($BW^{3/4}$) for oral data. Allometric scaling accounts for differences in physiological and biochemical processes, mostly related to kinetics.

For application of allometric scaling in risk evaluations, EPA uses dosimetric adjustment factors (DAFs), which can be calculated using Equation_Apx C-1.

Equation_Apx C-1. Dosimetric Adjustment Factor

$$DAF = \left(\frac{BW_A}{BW_H}\right)^{1/4}$$

Where:

DAF = Dosimetric adjustment factor (unitless)
BW_A = Body weight of species used in toxicity study (kg)
BW_H = Body weight of adult human (kg)

U.S. EPA (2011b), presents DAFs for extrapolation to humans from several species. However, because those DAFs used a human body weight of 70 kg, EPA has updated the DAFs using a human body weight of 80 kg for the *Draft Risk Evaluation for Phthalic Anhydride* (U.S. EPA, 2026i). EPA used the body weights of 0.025 kg for mice and 0.25 kg for rats, as presented in U.S. EPA (2011b). The resulting DAFs for mice and rats are 0.133 and 0.236, respectively.

Use of allometric scaling for oral animal toxicity data to account for differences among species allows EPA to decrease the default UF_A used to set the benchmark MOE; the default value of 10× can be decreased to 3×, which accounts for any toxicodynamic differences that are not covered by use of $BW^{3/4}$. Using the appropriate DAF from Equation_Apx C-1, EPA adjusts the POD to obtain the HED using Equation_Apx C-2:

Equation_Apx C-2. Daily Oral Human Equivalent Dose

$$HED_{Daily} = POD_{Daily} \times DAF$$

Where:

HED_{Daily} = Human equivalent dose assuming daily doses (mg/kg-day)
POD_{Daily} = Oral POD assuming daily doses (mg/kg-day)
DAF = Dosimetric adjustment factor (unitless)

For this draft risk evaluation, EPA determined that phthalic anhydride is a dermal and respiratory sensitizer, and therefore derived separate PODs for oral, dermal, and inhalation that are described in

Sections 4.1, 4.2, and 4.3, respectively. However, *o*-phthalic acid is not expected to have the same route-specific toxicity. Therefore, EPA assumed similar absorption for the oral and inhalation routes for *o*-phthalic acid, and no adjustment was made when extrapolating to the inhalation route. For the inhalation route, EPA extrapolated the daily oral HEDs to inhalation HECs using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest, as follows:

Equation_Apx C-3. Extrapolating from Oral HED to Inhalation HEC

$$HEC_{Daily,continuous} = HED_{Daily} \times \left(\frac{BW_H}{IR_R * ED_C}\right)$$

Where:

$HEC_{Daily,continuous}$	=	Inhalation HEC based on continuous daily exposure (mg/m^3)
HED_{Daily}	=	Oral HED based on daily exposure (mg/kg-day)
BW_H	=	Body weight of adult humans (kg) = 80
IR_R	=	Inhalation rate for an individual at rest (m^3/h) = 0.6125
ED_C	=	Exposure duration for a continuous exposure (h/day) = 24

Based on information from U.S. EPA (2011a), EPA assumes an at rest breathing rate of 0.6125 m^3/h. Adjustments for different breathing rates required for individual exposure scenarios are made in the exposure calculations, as needed.

It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in studies and the default units for different OPPT models. Therefore, EPA presents all PODs in equivalents of both units to avoid confusion and errors. Equation_Apx C-4 presents the conversion of the HEC from mg/m^3 to ppm.

Equation_Apx C-4. Converting Units for HECs (mg/m^3 to ppm)

$$X\ ppm = Y\ \frac{mg}{m^3} \times \frac{24.45}{MW}$$

Where:

24.45	=	Molar volume of a gas at standard temperature and pressure (L/mol), default
MW	=	Molecular weight of the chemical (MW of *o*-phthalic acid = 166.14 g/mol)

C.1 Phthalic Acid Non-Cancer HED and HEC Calculations for Intermediate and Chronic Duration Exposures

The chronic duration non-cancer POD used exclusively in the general population screening assessment is based on a NOAEL of 278 mg/kg-day, and the critical effect is decreased body weight at the LOAEL of 556 mg/kg-day. The POD was derived from a 2-year dietary study of male rats (NCI, 1979). This non-cancer POD is considered protective of effects observed following intermediate and chronic duration exposures to *o*-phthalic acid.

EPA used Equation_Apx C-1 to determine a DAF specific to rats (0.236), which was in turn used in the following calculation of the daily HED using Equation_Apx C-2:

$$66\ \frac{mg}{kg-day} = 278\ \frac{mg}{kg-day} \times 0.236$$

6007 EPA then calculated the continuous HEC for an individual at rest using Equation_Apx C-3:
6008
6009 $$358\ \frac{mg}{m^3} = 66\ \frac{mg}{kg-day} \times \left(\frac{80\ kg}{0.6125\ \frac{m^3}{hr} * 24\ hr}\right)$$
6010
6011 Equation_Apx C-4 was used to convert the HEC from mg/m^3 to ppm:
6012
6013 $$52.6\ ppm = 358\ \frac{mg}{m^3} \times \frac{24.45}{166}$$
6014

Appendix D SARA-ICE EXTENDED MODEL EXTRAPOLATION

D.1 Background and Summary

This appendix describes EPA's approach to apply additional EDx estimates from the SARA-ICE extended model to extrapolate to EDx values below 1% using the geometric means from the ED_{01}, ED_{05}, ED_{10}, and ED_{20} distributions. The purpose of this approach was to estimate the geometric mean (µg/cm^2) of the distribution at which the percent chance of inducing sensitization approaches zero, which could be used to inform the range in variability of the allergic response across the human population associated with exposure to phthalic anhydride.

EPA determined the EDx values for two values (*i.e.,* geometric mean (µg/cm^2) of the distribution): 4.5 and 15 µg/cm^2. The first reflects the effective dermal hazard value of 4.5 µg/cm^2 (*i.e.,* 45 µg/cm^2 divided by a total UF of 10×), and the second reflects the effective dermal hazard value of 15 µg/cm^2 (*i.e.,* 45 µg/cm^2 divided by a total UF of 3×). These values were selected to demonstrate a rough estimate of the protectiveness of the POD if additional UFs were applied.

As described in the analysis below, at an effective dermal hazard value of 4.5 µg/cm^2 or 15 µg/cm^2, the EDx is under 0.001%, indicating that over 99.99% of the population is protected.

D.2 Analysis

From the SARA-ICE extended model (Table_Apx D-1), EPA used the geometric means of the ED_{01}, ED_{05}, ED_{10}, and ED_{20} distributions to extrapolate EDx estimates to values below 1%. Specifically, EPA extrapolated to ED_{01} / 10 (based on an uncertainty factor of 10) and ED_{01} / 3 (based on an UF of 3).

The publicly-available SARA-ICE model does not yet provide uncertainty quantification (*e.g.,* standard error) for EDx values beyond the 5th and 95th percentile for ED_{01} (Reinke et al., 2026). Therefore, no statistical model could reliably be fit. Consequently, EPA extrapolated from the geometric means/median values that were provided for each EDx, but conventional goodness-of-fit statistical tests cannot be applied. In the absence of this, EPA instead used qualitative visual inspection of plotted fits to assess results.

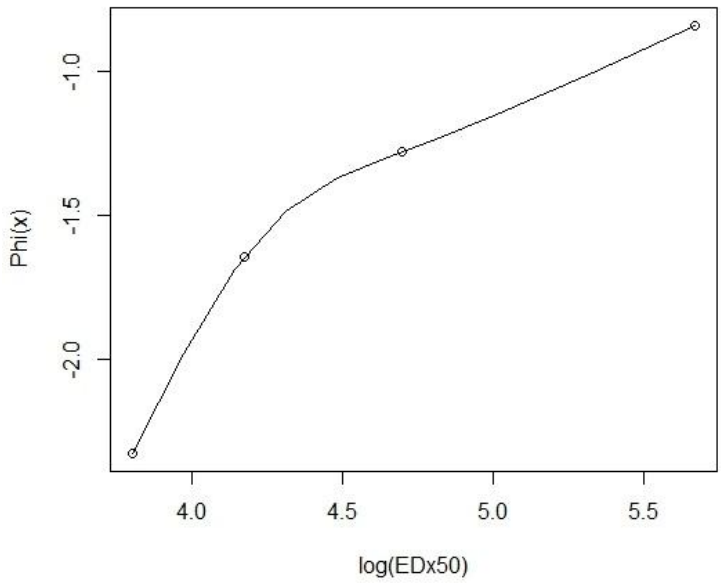
EPA used the R function "spline" from the base package "stats" to fit natural splines. Other fitting methods and models were also attempted, including exact cubic spline and local polynomial regression, however, these methods did not yield a reasonable quality of fit (qualitative assessment). EPA used multiple transformations of the data (*e.g.,* log, probit, and logit). Probit transformation involves converting a percentage to a real-valued number, based on the inverse cumulative density function (CDF) of the Gaussian distribution: $\Phi(x) = P(Normal(0,1) \leq x) = p$. Logit transformation also involves converting a percentage to a real-valued number, using the logit function: $logit(p) = \log\left(\frac{p}{1-p}\right)$. After the transformed percentages are fitted and extrapolated, the inverse probit and logit functions can convert them back into percentages.

The best fitting extrapolation results came from using natural splines to fit and extrapolate the log-transformed geometric means (logEDx) as the predictor and either a probit or logit transformation on the percentages. Figure_Apx D-1 shows the fitted splines for the probit transformation, and Figure_Apx D-2 shows the extrapolated values at EDx=15 and EDx=4.5. When inversely transformed back into percentages, the extrapolated values using probit represent less than 0.0002%. Figure_Apx D-3 shows the fitted splines for the logit transformation, and Figure_Apx D-4 shows the extrapolated values at

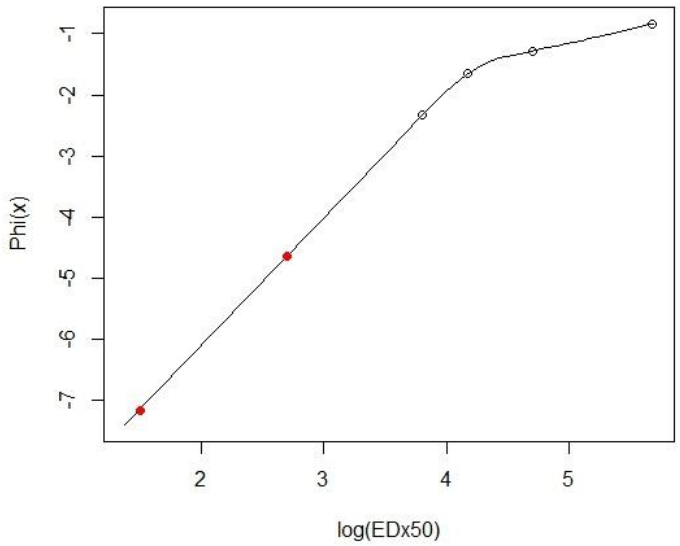
EDx=15 and EDx=4.5. When inversely transformed back into percentages, the extrapolated values using logit represent less than 0.004%.

Table_Apx D-1. Results of EDx Extrapolation

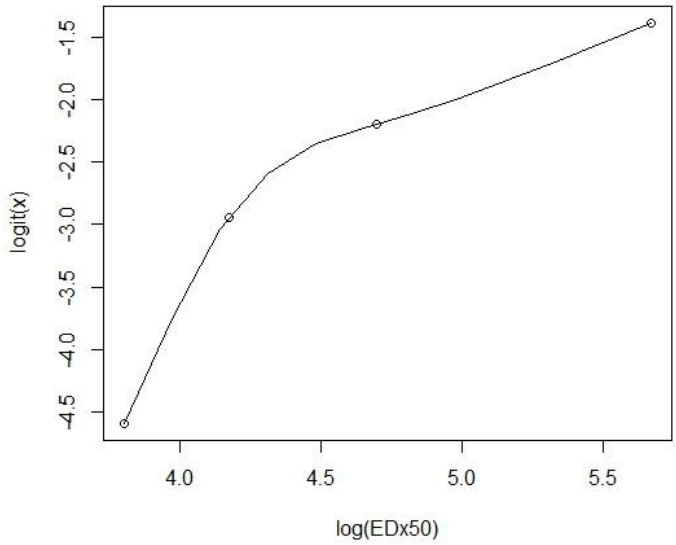
EDx Transform	Percentage Transform	Estimated % at EDx = 15	Estimated % at EDx = 4.5	Quality of Fit
No transform	No transform	−5.40%	−7.64%	Poor – extrapolation leads to negative % values
No transform	Probit	0.0260%	0.005%	Low – fit is not concave curve, as indicated by the data points
Log transform	Probit	0.00012%	<0.00001%	Good – fit smoothly follows the curvature of the data points
Log transform	Logit	0.0036	<0.00001%	Good – fit smoothly follows the curvature of the data points



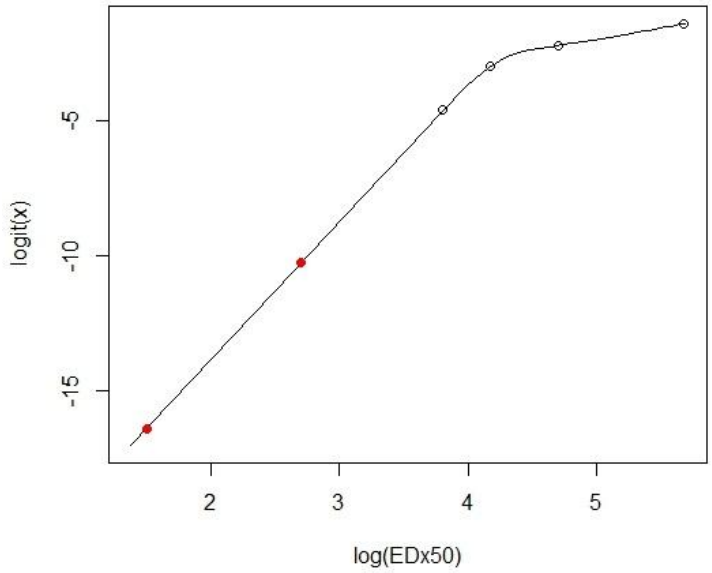
Figure_Apx D-1. Probit-Transformed Percentages and Log-Transformed Geometric Means with Fitted Natural Splines



Figure_Apx D-2. Probit Transformed Percentages and Log-Transformed Geometric Means with Extrapolated Values for 3× and 10× Uncertainty Factors



Figure_Apx D-3. Logit Transformed Percentages and Log-Transformed Geometric Means with Fitted Natural Splines



Figure_Apx D-4. Probit Transformed Percentages and Log-Transformed Geometric Means with Extrapolated Values for 3× and 10× Uncertainty Factors

Appendix E DOSIMETRIC ANALYSIS OF PHTHALIC ANHYDRYDE EXPOSURES

In this appendix, EPA describes the methods (Appendix E.2) and results (Appendix E.3) of dosimetric analyses of phthalic anhydride and TMA following inhalation exposure. These dosimetric analyses were considered in the dose-response analysis above in Section 4.3.1.3.1 for the candidate PODs from the Nielsen et al. (1988) study in exposed workers, the Sarlo et al. (1994) study of female Hartley guinea pigs, and the Leach et al. (1989) study of SD rats. This appendix includes the results of the characterization of human intersubject variability and an estimation of equivalent exposure concentrations in humans and animal models (Appendix E.3.2).

E.1 Background

In the respiratory tract, phthalic anhydride sensitization can affect both the upper airways and the lower airways. Described further below, Nielsen et al. (1988) reported phthalic anhydride exposure concentrations and durations for heavily exposed workers. Assuming that comparable doses within the respiratory tract should cause comparable effects within and across species, extrapolations only require that dose be defined and then characterized (Brown et al., 2005). EPA focused these analyses on the development of asthma and increases in airway responsiveness (*i.e.,* propensity for constriction of smooth muscles surrounding the tracheobronchial [TB] airways). An analysis was performed to evaluate potential interindividual variability in TB dose rates (µg/day) observed experimentally and caused by differences in breathing conditions, activity levels, and lung size of heavily exposed workers. Additionally, an interspecies comparison of TB doses in µg/day of phthalic anhydride or TMA per cm^2 of surface area was selected as the dose metric of interest for computation of exposure concentrations producing equivalent surface doses across species.

The exposure durations associated with sensitization ranged among studies from 5 days for guinea pigs to over a decade for workers. Daily doses for each weekday of exposure were calculated as a common exposure period among studies (*i.e.,* weekends without exposure were not considered). The daily exposure durations were 0.5 hours per workday for workers (Nielsen et al., 1988), 3 hours per weekday in guinea pigs (Sarlo et al., 1994), and 6 hours per weekday in rats (Leach et al., 1989). Estimating daily doses is like the use of an 8-hour TWA exposure to assess occupational exposures.

Nielsen et al. (1988) investigated the effects of phthalic anhydride in 35 heavily exposed workers relative to 25 workers having low exposures and 22 nonexposed control workers. Heavy exposures occurred once daily during 30-minute periods of dumping 25 kg phthalic anhydride bags into a reactor vessel. Cases of conjunctivitis, rhinitis, rhinoconjunctivitis, asthma, and chronic bronchitis were reported for the workers with low and heavy exposures. However, the statistical significance of those differences was not reported by the authors. Of the 35 heavily exposed workers and 25 workers with low exposures, Table_Apx E-1 provides the number of individuals experiencing symptoms and two-tailed statistical significance based on a two-sample proportion Z-test, Fisher's exact test, and the Chi-squared test. Based on uncertainties in the underlying distributions of the responses, preference was given to the Chi-squared results.

Conjunctivitis and asthma were significantly ($p \leq 0.05$) increased in workers with heavy phthalic anhydride exposures. There were a marginally significant effects ($0.05 < p \approx 0.10$) of heavy exposure on rhinitis and chronic bronchitis. For the five individuals with asthma, a latency period from the beginning of work to the debut of symptoms ranged from less than 0.5 to 18 years. The IgG (ELISA ratio) in those five with asthma (average, 4.6; range, 2.2–7.1) was significantly ($p = 0.005$) greater than in 26 individuals with no symptoms (average, 1.8; range, 0.6–5.5).

Table_Apx E-1. Respiratory Tract Symptoms in Nielsen et al. (1988) Study[a]

Symptom	Heavy Exposure Group[b]	Light Exposure Group[c]	Two-Sample Z-Test	Fisher's Exact Test	Chi-Squared Test
Conjunctivitis	16	5	**0.04**	**0.03**	**0.04**
Rhinitis	14	5	0.10	0.06	0.10
Rhinoconjunctivitis	6	3	0.58	0.25	0.59
Asthma	5	0	**0.05**	0.06	**0.05**
Chronic bronchitis	6	1	0.11	0.11	0.12
Total workers (N)	35	25	–	–	–

[a] p-values ≤ 0.05 are considered significant and are **bolded**.
[b] The heavy exposure group reflects workers categorized as reactor loaders experiencing a mean 30-minute peak of 6.6 mg/m^3 during manual loading of the reactors.
[c] The light exposure group reflects workers in the "other work" group.

The intersubject variability analyses (Appendix E.3.1) show that for workers, the upper 95% confidence bound for thoracic (*i.e.,* lung) exposures to phthalic anhydride aerosols is approximately 3 times greater than the central tendency estimate. The central tendency estimate was a conservative scenario of oronasal mouth breathers (*i.e.,* a fraction of each inhaled breath enters through the mouth even at rest) engaged in light exercise while dumping bags of phthalic anhydride into an open reactor vessel. The scenario was conservative in that a lower activity level such as sitting and nasal breathing would greatly reduce or eliminate thoracic exposures to phthalic anhydride aerosols. The estimates of equivalent exposure concentrations among humans and animals (Appendix E.3.2) show that for aerosol exposures, it is not adequate to only compare exposure concentrations among studies. Factors such as the particle size distribution and ventilatory intake rates can dramatically affect delivered doses. For example, the daily dose of inhaled particles to the TB region normalized to tissue surface area of rats exposed to a TMA aerosol at 53.5 µg/m^3 for 6 hours/day was nearly the same as the daily dose of guinea pigs exposed to a phthalic anhydride aerosol at 5,570 µg/m^3 for 3 hours/day.

E.2 Methods

The dosimetric analyses discussed herein had two primary goals: (1) characterize the human intersubject variability in TB dose rates that may occur among workers due to differences in breathing conditions, activity levels, and lung size; and (2) estimate equivalent exposure concentrations yielding the same TB surface dose of humans and animals. To achieve these goals, the respiratory deposition was estimated for workers exposed to phthalic anhydride aerosols in the Nielsen et al. (1988) study, SD rats experimentally exposed to TMA aerosols in the Leach et al. (1989) study, and guinea pigs experimentally exposed to phthalic anhydride aerosols in the Sarlo et al. (1994) study. For workers and rats, the fraction of particles depositing within regions of the respiratory tract (*i.e.,* the head; TB region, and pulmonary [PU] or alveolar region) was predicted by the publicly available MPPD model version 3.04 (©2016). As described later, an option to run the MPPD version 3.04 for guinea pigs was not available. The MPPD Model version 3.04 was recently used to estimate the deposition of lead-laden particles in the human respiratory tract (U.S. EPA, 2025) pursuant to the recommendation of an EPA Scientific Advisory Board peer review panel (U.S. EPA, 2020c). The deposition calculations by the MPPD model version 3.04 are identical to those of the EPA MPPD Model Software (MPPD EPA 2021 version 1.01) that was publicly released and peer reviewed in 2021. However, EPA's response to peer review of MPPD EPA 2021 version 1.01 was not completed as of February 2026. As a result, the latest

publicly available model (*i.e.,* version MPPD 3.04) was used for this assessment. Additional calculations of particle losses in the extrathoracic (ET; *i.e.,* the head) region of workers were performed based on the ICRP (1994) model to estimate the penetration of aerosols into the thorax.

The human exposure scenario was workers loading reactors with 25 kg bags of phthalic anhydride flakes for durations of approximately 30 minutes each day (Nielsen et al., 1988). Table 1 of Nielsen et al. (1988) provides results of 24 air samples collected at two plants. The overall average air concentration during reactor loading was 6.6 mg/m^3 (8-hour TWA of 0.4 mg/m^3). Measurable phthalic anhydride air concentrations (0.1–0.2 mg/m^3 or 1.5–3% of the initial concentration) were observed close to the reactors for up to 60 minutes. There were no data related to the particle size distribution for phthalic anhydride during reactor loading. The size of flakes was not reported. Internet searches of manufacture sites suggest the flakes are of variable widths of a few millimeters (mm) to a centimeter and relatively thin perhaps less than a mm. Based on reaching 1.5% of the initial air concentration at 60 minutes, EPA solved for the median aerodynamic diameter (MMAD) assuming a geometric standard deviation (GSD) of 1.6 that would achieve this change in concentration in 60 minutes. For stirred settling of an aerosol initially mixed to a height of 4 meters, a 19.5 µm MMAD (GSD = 1.6) aerosol reaches 1.5% of initial concentration at 60 minutes. The workplace MMAD would likely be larger since stirred settling does not account for the resuspension of particles caused by human activity or other industrial activities during the 60-minute period post reactor filling.

EPA also relied on Plinke M et al. (1995) estimates of dust generation from handling powders in industry. The objective of Plinke M et al. (1995) was to estimate the generation of respirable dust particles produced in industrial operations involving fall-type processes in which material fell through open air and landed on a pile of the same material. The process of dumping 25 kg bags of phthalic anhydride during reactor loading is a fall-type process in which material falls through open air and lands on a pile of the same material. Plinke M et al. (1995) reported that the amount of respirable particles in the original material influenced the amount of those particles generated as dust. Table 2 of Plinke M et al. (1995) provides experimentally measured particle size distributions coarse materials having aerodynamic diameters exceeding 25 µm. Based on those data, three potential particle size distributions for phthalic anhydride were considered: a 30 µm mass MMAD with a geometric standard deviation (GSD) of 1.6; a 50 µm MMAD with a GSD of 1.6; and 60 µm MMAD with a GSD of 1.9. Based on sedimentation rates, a 20 µm MMAD (GSD = 1.6) aerosol was also included in the characterization of intersubject variability.

The primary experimental animal exposure scenario was SD rats having whole body chamber exposures to TMA aerosols for 6 hours/day, 5 days/week, for 13 weeks (Leach et al., 1989). Particle sizes and chamber concentrations were obtained from Table 2 of IIT Research Institute (1988). There were four targeted chamber concentrations of 0, 2, 15, and 50 µg/m^3 which had 13-week TWA measured concentrations of 0.0, 2.2, 15.4, and 53.5 µg/m^3, respectively. At the target concentration of 2 µg/m^3, the experimental aerosol had a 1.74 µm MMAD with a GSD of 1.42. At the target concentrations of 15 and 50 µg/m^3, the experimental aerosol had a 2.2 µm MMAD with a GSD of 1.36. Weekly body weights of male and female rats were obtained from Tables 21 and 22, respectively, of IIT Research Institute (1988). On average, at the beginning of the 13-week study, female and male rats were 219 g and 312 g, respectively. After the 13-week study, females had gained an average of 93 g and males gained 226 g.

The secondary experimental animal exposure scenario was female Hartley guinea pigs (body weight, 350–400 g having whole body chamber exposures to phthalic anhydride aerosols for 3 hours/day for 5 days (Sarlo et al., 1994). In addition to filtered air exposures, there were three TMA targeted chamber concentrations of 0.5, 1.0, and 5.0 mg/m^3, which had 5-day TWA measured concentrations of 0.55, 1.27,

5.57 mg/m^3, respectively. With increasing exposure concentration, the experimental aerosols had MMAD (GSD) of 3.12 µm (2.02), 3.26 µm (1.96), and 3.91 µm (2.08). EPA was unnable to conduct dosimetric analyses for guinea pigs in a manner similar to the analyses completed for workers and SD rats using the MPPD Model. A draft MPPD version has been created to calculate deposition of particles from 0.3 to 10 µm in the lungs of guinea pigs (Asgharian, 2016). That draft model is currently being used by the Defense Threat Reduction Agency to predict regional deposition of *Bacillus anthracis* spore clusters found in particles of 1 to 12 µm in diameter. However, that functionality for guinea pigs is not available in either the MPPD version 3.04 or the MPPD EPA 2021 version 1.01. EPA instead relied on empirical particle deposition equations for guinea pigs (see Appendix G of U.S. EPA (1994)), which were derived from experimental data published by Raabe et al. (1988).

For a constant exposure concentration, breathing rate, and aerosol particle size distribution, the daily dose rate ($\dot{D}_r$) in µg/day of particles to region r of the respiratory tract is given by the following equation:

Equation_Apx E-1.

$$\dot{D}_r = DF_r \times C \times f\left(\frac{60\ min}{\boldsymbol{h}}\right) \times V_T\left(\frac{m^3}{10^6 mL}\right) \times t$$

Where:

DF_r	=	The deposition fraction of aerosol adjusted for inhalability in region r of the respiratory tract (dimensionless)
C	=	The mass concentration of the aerosol (µg/m^3)
f	=	Breathing frequency (min^{-1})
V_T	=	Tidal volume (mL)
t	=	The duration of exposure during a day of exposure (h).

Both the workplace and experimental aerosols described above had a GSD exceeding 1.1, indicating that they are polydisperse distributions. For polydisperse aerosols, the particle size distribution is broken into monodisperse intervals of equal mass or number, and DF_r for each interval is calculated. The overall DF_r for the polydisperse aerosol is found by the summation of monodisperse particle deposition fractions across all intervals divided by the number of intervals. For example, if a polydisperse aerosol is divided into 99 intervals of monodisperse aerosols (each corresponding to 1% of total aerosol mass), the overall or total DF_r of the polydisperse aerosol is estimated as summation of the DF_r across all the monodisperse intervals divided by 99.

The normalized daily dose rate ($N\dot{D}_r$) in µg/cm^2 per day is given by the following equation:

Equation_Apx E-2.

$$N\dot{D}_r = \dot{D}_r / SA_r$$

Where:

$\dot{D}_r$	=	The daily dose rate of particles to region r of the respiratory tract (µg/day).
SA_r	=	The surface area of region r of the respiratory tract (cm^2).

For cross-species comparisons, it is possible to solve for a rat exposure concentration that will give the same $N\dot{D}_r$ in rats as was estimated to occur in workers or vice versa. Because DF, f, V_T, t, and SA_r are constant for a given exposure scenario, the new exposure concentration (C_2) that will achieve a target $N\dot{D}_2$ is given by the following equation:

Equation_Apx E-3.

$$C_2 = C_1 \left({N\dot{D}_2}/{N\dot{D}_1} \right)$$

Where C_1 and $N\dot{D}_1$ are the original concentration and normalized surface dose, respectively. A detailed discussion of cross species dosimetric comparisons between rats and humans is available elsewhere (Brown et al., 2005).

E.2.1 Characterization of Human Intersubject Variability in TB Dose

E.2.1.1 Intersubject Variability Based on ICRP (1994)

The modeling approach in this appendix was previously used by EPA to determine the thoracic and respirable fraction of inhaled particles in male and female children and adults and published in a peer reviewed scientific publication (Brown et al., 2013). Thoracic and respirable fractions are defined as the fraction of inhaled particles capable of passing beyond the larynx and ciliated airways, respectively, during inhalation.

For a given inhaled particle size, variability in particle delivery to the TB region is caused by inter-individual differences in route of breathing and the deposition efficiency in the upper respiratory tract (*i.e.,* the nasal passages and the larynx). For a conservative (*i.e.,* relatively high delivered dose) baseline condition of an oronasal mouth breather, EPA estimated central tendency, upper- and lower-95% confidence interval bounds for inter-individual differences for males and females in particle penetration through the ET region for the activities of sitting, light exercise, and heavy exercise. Estimates were made for each of the four potential particle size distributions for phthalic anhydride (*i.e.,* 20 µm MMAD with a GSD of 1.6; 30 µm MMAD with a GSD of 1.6; 50 µm MMAD with a GSD of 1.6; 60 µm MMAD with a GSD of 1.9). For these large, inhaled particle sizes, the vast majority of particles penetrating the ET airways will deposit in the TB region. Thus, the particle penetration through ET region adjusted for particle inhalability can be substituted for the DF_{TB} in Equation_Apx E-1 to obtain an estimate of the daily dose rate to the TB region (*i.e.,* $\dot{D}_{TB}$).

For air passing through the mouth, deposition of large particles by impaction occurs mainly at the larynx. From Eq. D.30 of ICRP (1994), laryngeal deposition efficiency, $\eta(ET)_{larynx}$, is given by the following equation:

Equation_Apx E-4.

$$\eta(ET)_{larynx} = 1 - \{\alpha(1.1 \times 10^{-4})[d_a^2(Q_{total}SF_t^3)^{0.6}(V_T SF_t^3)^{-0.2}]^{1.4} + 1\}^{-1}$$

Where:

α	=	1 for a central tendency estimate or set to 0.30303 or 3.3 for lower- and upper-95% confidence bounds in modeled laryngeal deposition
d_a	=	Aerodynamic diameter (µm)
Q_{total}	=	Total inspiratory flow rate (mL/s)
V_T	=	Tidal volume (mL)
SF_t	=	A scaling factor of 1.0 for adult males and 1.08 for adult females from Table 15 of ICRP (1994).

The 95% confidence bound (also known as a 95% prediction interval) is expected to contain 95% of the observed and/or future data and is intended to characterize interindividual variability in particle

deposition. The ICRP (1994) specifically attributes interindividual variability in laryngeal deposition to "… differences in the morphology and physiology of this region, especially of the larynx and vocal cords."

For nasal breathing, ET deposition efficiency due to impaction was calculated from Eq. D.32 and D.33 of ICRP (1994) as provided in Equation_Apx E-5 and Equation_Apx E-6 below, respectively. The ET deposition efficiencies for the anterior, $h(ET_1)_{nose}$, and posterior, $h(ET_2)_{nose}$, nasal regions are given by Equation_Apx E-5 and Equation_Apx E-6:

Equation_Apx E-5.

$$\eta(ET_1)_{nose} = 0.5\,\{1 - [\alpha(3 \times 10^{-4})(d_a^2 Q_{nose} SF_t^3) + 1]^{-1}\}$$

Equation_Apx E-6.

$$\eta(ET_2)_{nose} = 1 - [\alpha(5.5 \times 10^{-5})(d_a^2 Q_{nose} SF_t^3)^{1.17} + 1]^{-1}$$

Where:

α = 1 for a central tendency estimate or set to 0.30303 or 3.3 for lower- and upper-95% confidence bounds in modeled nasal deposition

Q_{nose} = Inspiratory flow rate (mL/s) through the nose.

Like interindividual variability in laryngeal deposition, the 95% confidence bound characterizes interindividual variability in nasal particle deposition which is likely caused by morphometric and physiologic differences in nasal passages among individuals. The SF_t in Equation_Apx E-4 through Equation_Apx E-6 increases particle deposition efficiency by impaction with decreasing body size and increasing airflow resistance smaller airways.

The deposition fraction adjusted for inhalability of particles in the ET region, DF_{ET}, is given by

Equation_Apx E-7.

$$DF_{ET} = F_m\, I_m\, \eta(ET)_{larynx} + F_n\, I_n\, [\,\eta(ET_1)_{nose} + (1 - \eta(ET_1)_{nose})\,\eta(ET_2)_{nose}]$$

Where:

F_m and F_n = The fraction of air inhaled through the mouth and nose, respectively.

I_m and I_n = The inhalability of particles inhaled through the mouth (Brown, 2005) and nose (Ménache et al., 1995), respectively.

The penetration of particles through the ET region, P(ET), into the TB region is given by

Equation_Apx E-8.

$$P(ET) = F_m I_m\left[1 - \eta(ET)_{larynx}\right] + F_n I_n\{1 - [\eta(ET_1)_{nose} + (1 - \eta(ET_1)_{nose})\,\eta(ET_2)_{nose}]\}$$

In Equation_Apx E-4 through Equation_Apx E-6, an α of 0.30303 provides the lower 95% confidence bound for DF_{ET} in Equation_Apx E-7 and the upper 95% confidence bound for P(ET) in Equation_Apx E-8. As noted above, due to the large MMAD(s) being considered, the particle penetration through ET region adjusted for particle inhalability is approximately the DF_{TB}. Note, P(ET) cannot be calculated as one minus DF_{ET} due to particle inhalability. As expressed, Equation_Apx E-4 through Equation_Apx E-8 are for monodisperse aerosols. For polydisperse aerosols, the particle size distribution is broken into monodisperse intervals of equal mass or number, and the equations are calculated for each interval. The

overall estimate for a polydisperse aerosol is found by the summation of monodisperse particle estimates divided by the number of intervals.

E.2.1.1 Intersubject Variability Based on MPPD Simulations

Variability in particle doses to the TB region (*i.e.,* $\dot{D}_{TB}$ and $N\dot{D}_{TB}$) were also evaluated using the MPPD version 3.04 model for differences in breathing conditions (*i.e.,* route of breathing), activity levels (namely, sitting, light exercise, and heavy exercise), and lung size. This analysis was limited to males because similar effects would be observed in females. This analysis was also limited to the exposure scenario of a workplace aerosol having a 30 µm MMAD with a GSD of 1.6 at a concentration of 6.6 mg/m^3 for 30 minutes. Only the 30 µm MMAD aerosol, not the 50 or 60 µm MMAD aerosols, was considered in this analysis because it was expected to have greatest penetration into the lower respiratory tract. A conservative (*i.e.,* relatively high delivered dose) baseline condition of an oronasal mouth breather engaged in light exercise was used. The activity level of light exercise was assumed to represent the 30-minute periods of reactor loading with 25 kg bags of phthalic anhydride.

For an individual engaged in light exercise, breathing conditions were varied by route of breathing from the baseline of oronasal mouth breathing (57% oral and 43% nasal) to 100% oral and 100% nasal breathing. For an oronasal normal augmenter engaged in light exercise, air is inhaled 100% via the nose. From Table B.15 of ICRP (1994), breathing patterns, V_T and f, as a function of activity were 1250 mL (20 min^{-1}) for light activity, 750 mL (12 min^{-1}) for sitting, and 1,923 mL (26 min^{-1}) for heavy exercise. From Table 15 of ICRP (1994), a baseline functional residual capacity (FRC, the lung volume at the end of a normal exhalation during tidal breathing) of 3,300 mL and an upper respiratory tract (URT) volume of 50 mL were assumed. The URT volume varied by ±20%. The baseline FRC was varied by ±650 mL to represent 10th to 90th percentiles of height for nonsmoking, white males between 18 and 35 years of age McDonnell and Seal (1991). For MPPD estimates of particle deposition, the Yeh/Schum Symmetric morphology was selected. The inhaled aerosol was adjusted for particle inhalability from calm air for nasal breathing from Ménache et al. (1995) and for oral breathing from Brown (2005).

E.2.2 Estimation of Equivalent Cross Species Exposure Concentrations

An interspecies comparison of TB dose rates in µg/day of phthalic anhydride or TMA per cm^2 of surface area (*i.e.,* $N\dot{D}_{TB}$) was selected as the dose metric of interest for computation of exposure concentrations producing equivalent surface doses across species. Since the exposure period of interest was only 0.5 hours per workday in workers (Nielsen et al., 1988), 3 hours per weekday in guinea pigs (Sarlo et al., 1994), and 6 hours per weekday in rats (Leach et al., 1989), daily surface doses were calculated for each day of exposure.

For workers, the conservative (*i.e.,* relatively high delivered dose) baseline condition of an oronasal mouth breather engaged in light exercise was used. From Table 15 of ICRP (1994), FRC for females and males were 2,681 mL and 3,300 mL, respectively; URT volumes for females and males were 40 mL and 50 mL, respectively. From Table B.15 of ICRP (1994), light activity corresponds to V_T and f of 992 mL (21 min^{-1}) for females and 1250 mL (20 min^{-1}) for males. As described above, the exposure was 30 minutes to a concentration of 6.6 mg/m^3 and three workplace aerosols were a 30 µm MMAD with a GSD of 1.6, a 50 µm MMAD with a GSD of 1.6, and 60 µm MMAD with a GSD of 1.9. For MPPD estimates of particle deposition, the Yeh/Schum Symmetric morphology was selected. The three aerosols were adjusted for particle inhalability from calm air for nasal breathing from Ménache et al. (1995) and for oral breathing from Brown (2005).

For rats, a symmetric SD morphology was selected which was scaled based on body weight in grams. Although the rat lung is asymmetric, the symmetric morphology allows for clear resolution deposition

within the TB airways from that within PU region. The lack of an overlap in body weight between the sexes necessitated calculations be performed separately for male and female rats. Body weight is used by the MPPD to predict functional residual capacity (FRC) based on Takezawa et al. (1980). The V_T and *f* for whole body exposures (as opposed to nose only) were estimated based on Miller et al. (2014). Regardless of species, lower respiratory tract airway dimensions (length and diameter) are isotropically (*i.e.,* indifferent to direction) scaled to FRC plus 0.5 × V_T for deposition calculations. The aerosols were adjusted for particle inhalability of small laboratory animals (Ménache et al., 1995).

For guinea pigs, the particle deposition efficiency (η) in region r of the respiratory tract is given by

Equation_Apx E-9.

$$\eta_r = \frac{1}{1 + e^{\alpha + \beta \log x}}$$

Where α and β are 2.253 and −1.282 for the ET region, 2.522 and −0.865 for the TB region, and 0.754 and 0.556 for the PU region, respectively; and x is an impaction parameter, which equals d_a^2 (in µm) ×inspiratory flow rate (in mL/s) for the ET region and d_a for the TB and PU regions (Section G.2 of U.S. EPA (1994)). The inspiratory flow was assumed to be 6.11 mL/s (Eq. 4-4 of U.S. EPA (1994)). The deposition fraction (DF) for each region of the respiratory tract is given by the following equations:

Equation_Apx E-10.

$$DF_{ET} = I_{animal} \times \eta_{ET}$$

Equation_Apx E-11.

$$DF_{TB} = I_{animal} \times (1 - \eta_{ET}) \times \eta_{TB}$$

Equation_Apx E-12.

$$DF_{PU} = I_{animal} \times (1 - \eta_{ET}) \times (1 - \eta_{TB}) \times \eta_{PU}$$

Where:

I_{animal} = An inhalability adjustment for small laboratory animals (Ménache et al., 1995).

Assuming an allometric adjustment root of 1/3, the DF_{TB} was normalized to a TB surface area of 171 cm^2 for 375 g guinea pigs based on a TB surface area of 200 cm^2 in 600 g guinea pigs (Schreider and Hutchens, 1980). As expressed, Equation_Apx E-9 through Equation_Apx E-12 are for monodisperse aerosols. For polydisperse aerosols, the particle size distribution is broken into monodisperse intervals of equal mass or number, and the equations are calculated for each interval. The overall estimate for a polydisperse aerosol is found by the summation of monodisperse particle estimates divided by the number of intervals.

E.3 Results and Discussion

E.3.1 Characterization of Human Intersubject Variability in TB dose

E.3.1.1 Intersubject Variability Based on ICRP (1994)

Table_Apx E-2 provides central tendency estimates of DF_{ET} based on the ICRP (1994) for comparison with those calculated by the MPPD model which are provided later for light exercise in Table_Apx E-7.

Table_Apx E-2. Particle Deposition in the Extrathoracic Region of Workers as a Function of Activity and Particle Size Based on ICRP (1994)

Sex	Activity	20 µm MMAD	30 µm MMAD	50 µm MMAD	60 µm MMAD
Male	Sitting	0.672	0.535	0.336	0.292
Female	Sitting	0.665	0.498	0.281	0.244
Male	Light Exercise	0.727	0.622	0.446	0.388
Female	Light Exercise	0.726	0.614	0.433	0.377
Male	Heavy Exercise	0.755	0.635	0.481	0.419
Female	Heavy Exercise	0.758	0.652	0.478	0.416

MMAD = mass median aerodynamic diameter

Table_Apx E-3 shows the penetration of particles through the ET region (see Equation_Apx E-7). Three factors caused females to have less P(ET) than males. First, increased particle losses in the head of females relative to males occurs due to the SF_t of 1.08 for females in Equation_Apx E-4 through Equation_Apx E-6, which increases losses in the smaller URT volume of females relative to males. The lower ET penetration of females than males is also due to having a greater fraction of air inhaled through the nose which has a greater particle deposition efficiency than the mouth. Additionally, the higher fraction of nasal breathing of females decreased the inhalability of all three aerosols at all three activity levels relative to males.

Table_Apx E-3. Percent of Particles Penetrating through the Extrathoracic Region of Workers as a Function of Activity and Particle Size

Sex	Activity	20 µm MMAD	30 µm MMAD	50 µm MMAD	60 µm MMAD
Male	Sitting	6.0 (2.2, 14) [a]	2.3 (0.8, 6.0)	0.5 (0.2, 1.6)	0.6 (0.2, 1.7)
Female	Sitting	4.3 (1.6, 11)	1.6 (0.5, 4.4)	0.4 (0.1, 1.1)	0.4 (0.1, 1.2)
Male	Light Exercise	5.1 (1.8, 12)	1.8 (0.6, 5.1)	0.4 (0.1, 1.3)	0.5 (0.1, 1.4)
Female	Light Exercise	4.6 (1.6, 11)	1.6 (0.5, 4.6)	0.4 (0.1, 1.2)	0.4 (0.1, 1.3)
Male	Heavy Exercise	3.8 (1.3, 9.8)	1.3 (0.4, 3.8)	0.3 (0.1, 0.9)	0.3 (0.1, 1.0)
Female	Heavy Exercise	3.2 (1.1, 8.7)	1.1 (0.4, 3.3)	0.2 (0.1, 0.8)	0.3 (0.1, 0.9)

MMAD = mass median aerodynamic diameter.
[a] Values are a central tendency estimate with the 95% confidence bounds in parentheses.

Based on Table_Apx E-3, the upper 95% confidence bound for P(ET) is 2.9 times (range 2.3–3.2) greater than the central tendency (or arithmetic mean) estimate. The 95% confidence bound (also known as 95% prediction interval) is expected to contain 95% of the observed and/or future data. For the aerosols being modeled, the vast majority of particles penetrating the head will deposit in the TB region (*i.e.,* P[ET] approximately equals DF_{TB}). The lower and upper 95% confidence bounds of P(ET) are the 2.5 and 97.5 percentiles of DF_{TB}, respectively. Assuming a lognormal distribution of DF_{TB} among individuals, the geometric mean DF_{TB} and GSD can be calculated based on the 2.5 and 97.5 percentiles of DF_{TB}. The upper 95% confidence bound for DF_{TB} is approximately 3 times greater than the central tendency estimate. Using the geometric mean DF_{TB} and the GSD it is calculated that, on average, 44% of observed DFTB for both males and females are expected between the central tendency DF_{TB} estimate and the 95% upper confidence bound. The fraction of individual DF_{TB} withing a factor of 3 times around the geometric mean DF_{TB} can also be estimated. A factor of 3 times change in DF_{TB} above and below the geometric mean DF_{TB} is given by the geometric mean DF_{TB} multiplied and divided by 1.73 (*i.e.,* the square root of 3), respectively. On average, for the scenarios in Table_Apx E-3, a 3 times change in DF_{TB} around the geometric mean DF_{TB} is expected to capture 68% (range, 64–76%) of future observed

DF_{TB} in males and to capture 68% (range, 64–74%) of future observed DF_{TB} in females. The upper 95% confidence bound for DF_{TB} is approximately 3 times greater than the central tendency estimate. Interindividual variability in ET particle deposition and penetration is likely caused by morphometric and physiological differences in the ET airways among individuals. Thus, a factor of three (3) accounts for interindividual (*i.e.,* intraspecies) differences in doses delivered to the lower respiratory tract between EPA's central tendency and upper confidence bound estimates.

E.3.1.2 Intersubject Variability Based on MPPD Simulations

Table_Apx E-4 shows effect of route of breathing on the $\dot{D}_r$ in males during light exercise. Relative to an oronasal mouth breather, $\dot{D}_{TB}$ was increased by only 13% in the unrealistic case of pure oral breathing and decreased by 98% by nasal breathing. The nearly complete removal of particles in the nasal passages is consistent with Brown et al. (2013) who reported that the penetration of particles greater than 1 μm MMAD into the lower respiratory tract of humans was more affected by route of breathing than age, sex, activity level, or breathing pattern (*i.e.,* V_T and f). Since the TB surface area at the end of a normal breath (*i.e.,* FRC) is not affected by route of breathing, changes in $N\dot{D}_{TB}$ are identical to changes in $\dot{D}_{TB}$ in Table_Apx E-4.

Table_Apx E-4. Effect of Route of Breathing on Regional Deposition Fractions and Daily Dose Rates (μg/day) in Males During Light Exercise

Region	$DF_{oronasal}$	$\dot{D}_{oronasal}$	DF_{oral}	$\dot{D}_{oral}$	DF_{nasal}	$\dot{D}_{nasal}$
ET	0.611	3,022	0.746	3,690	0.452	2238
TB[a]	2.59E−02	128 (0%)	2.93E−02	145 (+13%)	4.65E−04	2.30 (−98%)
PU	1.12E−03	5.52	1.35E−03	6.69	7.14E−05	0.35

Notes: $DF_{oronasal}$, DF_{oral}, DF_{nasal} = deposition fraction for oronasal mouth breather, 100% oral breathing, and 100% nasal breathing, respectively; $\dot{D}_{oronasal}$, $\dot{D}_{oral}$, $\dot{D}_{nasal}$ = daily dose rate for oronasal mouth breather; 100% oral breathing, and 100% nasal breathing, respectively; ET = extrathoracic; TB = tracheobronchial; PU = pulmonary
[a] Parenthetical values are the percent change from an oronasal mouth breather.

Table_Apx E-5 shows effect of activity level on the $\dot{D}_r$ in males during light exercise. The data in this table emphasize the importance of considering $\dot{D}_r$, because it is directly proportional to the product of V_T and f, rather than simply looking at deposition fractions. For example, relative to light exercise, the deposition fraction in the TB region increased by 35% for sitting and decreased by 26% for heavy exercise. However, relative to light exercise, $\dot{D}_{TB}$ decreased by 51% for sitting and increased by 48% by heavy exercise. There is 1.5 times greater delivery of particles to the TB region when going from the base case of light exercise to heavy exercise. However, a broader absolute change in $\dot{D}_{TB}$ as a function of activity level occurs when going from sitting to heavy exercise which increased the dose by 200% (*i.e.,* a 3 times increase). Thus, a factor of one and a half (1.5) or a factor of three (3) accounts for inter-individual differences in doses delivered to the TB region depending on whether sitting at rest or light exercise is considered as the base scenario. Because the TB surface area at the end of a normal breath (*i.e.,* FRC) is not affected by activity level, changes in $N\dot{D}_{TB}$ are identical to changes in $\dot{D}_{TB}$ in Table_Apx E-5.

Table_Apx E-5. Effect of Activity Level with Oronasal Mouth Breathing on Daily Dose (µg/day) Regions of the Male Respiratory Tract

Region	$DF_{LightEx}$	$\dot{D}_{LightEx}$	$DF_{sitting}$	$\dot{D}_{sitting}$	DF_{HeavEx}	$\dot{D}_{HeavyEx}$
ET	0.611	3022	0.518	924	0.645	6385
TB*	2.59E−02	128 (0%)	3.50E−02	62.4 (−51%)	1.92E−02	190 (+48%)
PU	1.12E−03	5.52	1.63E−03	2.91	1.77E−04	1.75

$DF_{LighttEx}$, $DF_{sitting}$, $DF_{HeavyEx}$ = deposition fraction for oronasal mouth breather, 100% oral breathing, and 100% nasal breathing, respectively; $\dot{D}_{LighttEx}$, $\dot{D}_{sitting}$, $\dot{D}_{HeavyEx}$ = daily dose rate for oronasal mouth breather, 100% oral breathing, and 100% nasal breathing, respectively; ET = extrathoracic; TB = tracheobronchial; PU = pulmonary
*Parenthetical values are the percent change from an oronasal mouth breather.

Table_Apx E-6 shows effect of FRC on the $\dot{D}_r$ in males during light exercise with oronasal mouth breathing. Relative to an FRC of 3300 mL, a 650 mL reduction in FRC caused a 2% increase in $\dot{D}_{TB}$, whereas a 650 mL increase in FRC caused a 2% decrease in $\dot{D}_{TB}$. The TB surface area is calculated at FRC. A 650 mL reduction in FRC caused an 18% increase in $N\dot{D}_{TB}$, whereas a 650 mL increase in FRC caused a 13% decrease in $N\dot{D}_{TB}$.

Changing the URT volume had minimal effects on the $\dot{D}_r$. Changing the URT by 20% (*i.e.,* 10 mL) caused no effect on MPPD predicted losses in the head. This was unexpected and is inconsistent with the expectation that particle deposition efficiency by impaction will increase with decreasing body size and increasing airflow resistance through smaller airways. Decreasing the URT increased $\dot{D}_{TB}$ and $\dot{D}_{PU}$ by approximately 1%. Conversely, increasing the URT decreased $\dot{D}_{TB}$ and $\dot{D}_{PU}$ by approximately 1%. Since the TB surface area at the end of a normal breath (*i.e.,* FRC) is not affected URT volume, changes in $N\dot{D}_{TB}$ are identical to changes in $\dot{D}_{TB}$.

Table_Apx E-6. Effect of Function Residual Capacity on Daily Dose (µg/day) to Regions of the Male Respiratory Tract

Region	$DF_{3300\ mL}$	$\dot{D}_{3300\ mL}$	$DF_{2650\ mL}$	$\dot{D}_{2650\ mL}$	$DF_{3950\ mL}$	$\dot{D}_{3950\ mL}$
ET	0.611	3022	0.611	3022	0.611	3023
TB[a]	2.59E−02	128 (0%)	2.65E−02	131 (+2%)	2.54E−02	126 (−2%)
PU	1.12E−03	5.52	7.45E−04	3.89	1.40E−03	6.91

$DF_{3300\ mL}$, $DF_{2650\ mL}$, $DF_{3950\ mL}$, deposition fraction for oronasal mouth breather, 100% oral breathing, and 100% nasal breathing, respectively; $\dot{D}_{3300\ mL}$, $\dot{D}_{2650\ mL}$, $\dot{D}_{3950\ mL}$, daily dose rate for oronasal mouth breather, 100% oral breathing, and 100% nasal breathing, respectively; ET = extrathoracic; TB = tracheobronchial; PU = pulmonary
[a] Parenthetical values are the percent change from an oronasal mouth breather.

The largest increase in $\dot{D}_{TB}$ relative to an oronasal mouth breather engaged in light exercise occurred for heavy exercise which caused a 48% increase (1.5 times greater) in $\dot{D}_{TB}$. The largest absolute change in $\dot{D}_{TB}$ as a function of activity level was going from sitting to heavy exercise which increased the dose by 200% (3-fold increase). However, assessing the change from sitting to heavy activity is not necessarily consist with the baseline case of light exercise within this appendix. Switching from light activity to sitting decreases $\dot{D}_{TB}$ by 2.1-fold. Switching to oral breathing only resulted in a 13% increase (1.1-fold greater) in $\dot{D}_{TB}$. For the large aerosol (30 µm MMAD) being considered, switching from oronasal mouth breathing to nasal breathing prevented most particles from penetration into the lower respiratory tract and nearly eliminated the $\dot{D}_{TB}$. This demonstrates why oronasal mouth breathing was characterized as a conservative (*i.e.,* relatively high delivered dose) condition of exposure. Overall, MPPD simulations support an intersubject variability a factor of 1.5 to 3.0, depending on range in activity levels considered.

A factor of three (3) to account for interindividual differences in doses delivered to the lower respiratory tract was supported by ICRP (1994) based simulations in Appendix E.3.1.1.

E.3.2 Estimation of Equivalent Cross-Species Exposure Concentrations

In the Nielsen et al. (1988) study, workers were exposed to 6.6 mg/m^3 of phthalic anhydride for 30 minutes while loading reactors with 25 kg bags. The exposure concentration is equivalent to an 8-hour TWA of 0.41 mg/m^3 from 0.5 hours at 6.6 mg/m^3 and the remaining 7.5 hours at 0.0 mg/m^3. The particle sizes that workers inhaled were almost entirely (>99%) greater than 10 µm in aerodynamic diameter. For females, the 30, 50, and 60 µm MMAD aerosols were 62.9, 43.7, and 38.1% inhalable, respectively. For males, the 30, 50, and 60 µm MMAD aerosols were 63.9, 45.0, and 39.2% inhalable, respectively. The slightly greater aerosol inhalability for males is due to 57% of the breath entering through the mouth verses 54% for females. Of the inhaled particles, the large particle size results in the majority of particle deposition occuring in the head with only a small fraction of particles entering and depositing in the lower respiratory tract (Table_Apx E-7). The DF_{ET} in Table_Apx E-7 are quite similar to that Table_Apx E-2 for light exercise. For the 30 µm MMAD aerosol, Table E-7 shows DE_{ET} of 0.611 (males) and 0.599 (females), whereas Table_Apx E-2 shows DE_{ET} of 0.622 (males) and 0.614 (females). The resulting $N\dot{D}_{TB}$ estimated to occur each workday are provided in Table_Apx E-8.

Table_Apx E-9 is included to demonstrate the effect smaller particle sizes (MMAD of 5 and 10 µm with GSD of 2.5) on dose rates that may be associated with exposures from other types of activity such as spraying operations. For facilitate comparison with Table_Apx E-5 and Table_Apx E-6, the results in Table_Apx E-9 are for workers exposed to 6.6 mg/m^3 of phthalic anhydride for 30 minutes during light exercise. Relative to the 30 µm MMAD aerosol in Table_Apx E-8, the $N\dot{D}_{TB}$ for males increased by 2.6-fold for the 10 µm MMAD aerosol and 3.9-fold for the 5 µm MMAD aerosol.

Table_Apx E-7. Regional Particle Deposition Fractions in Workers

Sex	MMAD (µm)	GSD	DF_{ET}	DF_{TB}	DF_{PU}
Male	30	1.6	0.611	2.6E−02	1.1E−03
Female	30	1.6	0.559	2.8E−02	1.1E−03
Male	50	1.6	0.443	6.8E−03	3.8E−05
Female	50	1.6	0.429	7.3E−03	3.8E−05
Male	60	1.9	0.384	7.7E−03	2.8E−04
Female	60	1.9	0.372	8.2E−03	2.8E−04

MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation; DF_r = particle deposition fraction in region r of the respiratory tract; ET = extrathoracic; TB = tracheobronchial; PU = pulmonary

Table_Apx E-8. Daily Tracheobronchial Surface Dose (µg/day/cm^2) in Workers

MMAD (µm)	Males	Females	Average
30	3.1E−02	3.8E−02	3.4E−02
50	7.6E−03	1.0E−02	8.8E−03
60	8.6E−03	1.1E−02	9.9E−03

MMAD = mass median aerodynamic diameter

Table_Apx E-9. Regional Particle Deposition Fractions and Daily Tracheobronchial Surface Dose (µg/day/cm^2) for Smaller Aerosols in Male Workers

Sex	MMAD (µm)	DF_{ET}	DF_{TB}	DF_{PU}	$N\dot{D}_{TB}$
Male	5	0.483	1.1E−01	1.2E−01	1.2E−01
Female	5	0.479	1.1E−01	1.2E−01	1.2E−01
Male	10	0.586	7.2E−02	1.2E−01	8.1E−02
Female	10	0.583	7.4E−02	1.2E−01	7.9E−02

MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation; DF_r = particle deposition fraction in region r of the respiratory tract; ET = extrathoracic; TB = tracheobronchial; PU = pulmonary; $N\dot{D}_{TB}$ = daily TB dose rate normalized to TB surface area

For the Leach et al. (1989) rat study, 99% of the inhaled particles were less than 5 µm in diameter. The aerosol was 86.1% inhalable at the target concentration of 2 µg/m^3 and 82.6% inhalable at the higher target concentrations. Table_Apx E-10 provides the regional deposition fractions for inhaled particles. A considerably larger fraction of inhaled particles reached and deposited in the lower respiratory tract of rats (see DF_{ET} and DF_{TB} in Table_Apx E-10) relative to workers (see DF_{ET} and DF_{TB} in Table_Apx E-7). Table_Apx E-11 provides the resulting $N\dot{D}_{TB}$ estimated to occur each exposure day in rats at 6.5 weeks of exposure. Due to the differences in aerosol size distributions between exposure concentrations, the $N\dot{D}_{TB}$ in rats increased by 9 times as concentration increased by 7 times from 2.2 to 15.4 µg/m^3.

Table_Apx E-10. Regional Particle Deposition Fractions at 6.5 Weeks in Rats

Sex	MMAD (µm)	GSD	DF_{ET}	DF_{TB}	DF_{PU}
Male	1.74	1.42	0.231	0.119	0.041
Female	1.74	1.42	0.215	0.118	0.034
Male	2.2	1.36	0.276	0.144	0.041
Female	2.2	1.36	0.232	0.148	0.036

MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation; DF_r = particle deposition fraction in region r of the respiratory tract; ET =extrathoracic; TB =tracheobronchial; PU = pulmonary

Table_Apx E-11. Daily Tracheobronchial Surface Dose (µg/day/cm^2) at 6.5 Weeks in Rats

Exposure (µg/m^3)	Males	Females	Average
2.2	2.7E−03	2.2E−03	2.5E−03
15.4	2.4E−02	2.1E−02	2.2E−02
53.5	8.3E−02	7.2E−02	7.7E−02

MMAD = mass median aerodynamic diameter

For the Sarlo et al. (1994) study of guinea pigs, at the exposure concentrations of 0.55 and 1.27 mg/m^3, 74 to 75% aerosol particles were less than 5 µm in diameter, and 73 to 74% were inhalable. At the highest target concentration of 5.57 mg/m^3, 63% of the aerosol particles were less than 5 µm in diameter, and 69% were inhalable. Table_Apx E-12 provides the regional deposition fractions for inhaled particles and daily $N\dot{D}_{TB}$. By comparison of the highest exposure concentrations in Table_Apx E-11 for rats and Table_Apx E-12 for guinea pigs, it is informative to see that the $N\dot{D}_{TB}$ for rats is 1.04

times greater than for guinea pigs despite guinea pigs being exposed to a 104 times greater concentration (5,570 vs. 53.5 µg/m^3). To match the high exposure $N\dot{D}_{TB}$ of rats and guinea pigs, the guinea pig exposure would be increased from 5,570 to 5,770 µg/m^3. Similarly at the mid-concentrations, the $N\dot{D}_{TB}$ for rats is 1.2 times greater than for guinea pigs despite guinea pigs being exposed to an 82 times greater concentration (1,270 vs. 15.4 µg/m^3). To match the mid-concentration exposure $N\dot{D}_{TB}$ of rats and guinea pigs, the guinea pig exposure would be increased from 1,270 to 1,510 µg/m^3. The lowest exposure concentration for rats of 2.2 µg/m^3 is 250 times lower than 550 µg/m^3 for guinea pigs, yet the $N\dot{D}_{TB}$ is only 3.3 times lower in rats than guinea pigs. To match the lowest exposure $N\dot{D}_{TB}$ of rats and guinea pigs, the guinea pig exposure would be decreased from 550 to 160 µg/m^3. These similarities in $N\dot{D}_{TB}$ between rats and guinea pigs emphasize the need, where possible, to carefully consider differences exposure scenarios (namely, inhaled particle sizes and species differences)—not simply pollutant concentration. That is, for aerosol exposures it is not adequate to only compare exposure concentrations among studies because other factors such as the particle size distribution and ventilatory intake rates can dramatically affect delivered doses.

Table_Apx E-12. Regional Particle Deposition Fraction and Daily Tracheobronchial Surface Dose (µg/day/cm^2) for in Female Guinea Pigs

Concentration (mg/m^3)	MMAD (µm)	GSD	DF_{ET}	DF_{TB}	DF_{PU}	$N\dot{D}_{TB}$
0.55	3.12	2.02	0.348	0.039	0.098	8.3E−02
1.27	3.26	1.96	0.354	0.038	0.094	1.9E−02
5.57	3.91	2.08	0.356	0.035	0.081	7.5E−02

MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation; DF_r = particle deposition fraction in region r of the respiratory tract; ET = extrathoracic; TB = tracheobronchial; PU = pulmonary; $N\dot{D}_{TB}$ = daily tracheobronchial surface dose

Although the particle size distribution, daily exposure durations, and exposure concentrations were vastly different between the workers and animal studies, it was possible to solve for an animal exposure concentration (Equation_Apx E-3) giving the same $N\dot{D}_r$ in animals as was estimated for workers. Since there were differences in the $N\dot{D}_{TB}$ between the sexes for both species but in opposite directions, concentration estimates for equivalent $N\dot{D}_{TB}$ were made using the average of females and males for each species. For rat equivalent concentrations, it was assumed that the rats were exposed 1.74 µm MMAD (GSD=1.42) if the concentration was <15.4 µg/m^3 and 2.2 µm MMAD (GSD=1.36) if the concentration was ≥15.4 µg/m^3. For guinea pig equivalent concentrations, it was assumed that the rats were exposed 3.12 µm MMAD (GSD=2.02) and/or 3.26 µm MMAD (GSD=1.96). Table_Apx E-13 provides the equivalent exposure concentrations that rats (average of males and females) would need to be exposed to for 6 hours at 6.5 weeks of exposure to produce the estimated $N\dot{D}_{TB}$ of workers (average of males and females) exposed to aerosols of 30, 50, or 60 µm MMAD. The estimated rat equivalent exposure concentrations (range 7.85–23.7 µg/m^3) were within experimental range (2.2–53.5 µg/m^3) investigated by the Leach et al. (1989) study of rats. Table_Apx E-14 provides the equivalent exposure concentrations that guinea pigs (females) would need to be exposed to for 3 hours a day to produce the estimated $N\dot{D}_{TB}$ of workers (average of males and females) exposed to a 6.6 mg/m^3 aerosol with either a 30, 50, or 60 µm MMAD. The estimated guinea pig equivalent to human exposure concentrations (range 0.59–2.30 mg/m^3) were within experimental range (0.55–5.57 mg/m^3) investigated by the Sarlo et al. (1994) study of guinea pigs.

The lowest rat exposure concentration of TMA shown to produce effects at 6.5-weeks is 2.2 µg/m^3 with exposures of 6 hours/day and 5 days/week (Leach et al., 1989). Table_Apx E-15 provides the human

equivalent exposure concentration for workers having exposures of 30 minutes/workday to varied aerosols (MMAD of 30, 50, and 60 µm) matching the $N\dot{D}_{TB}$ estimated for rats exposed to 2.2 µg/m^3 of aerosol (1.74 µm MMAD, 1.42 GSD) with exposures of 6 hours/day. Likewise, Table_Apx E-16 provides the human equivalent exposure concentration for workers having exposures of 30 minutes/workday to varied aerosols (MMAD of 30, 50, and 60 µm) matching the $N\dot{D}_{TB}$ estimated for guinea pigs exposed to 0.55 mg/m^3 of aerosol (3.12 µm MMAD, 2.02 GSD) with exposures of 3 hours/day. Should future experimental animal studies become available that identify a no effects exposure scenario, the approaches described herein can be used to translate the animal exposure scenario to a human equivalent exposure scenario.

Table_Apx E-13. Rat Experimental 6-Hour/Day Exposure Concentrations Producing Tracheobronchial Surface Doses Equivalent to Workers Exposed 0.5 Hours/Day

Workplace MMAD (µm)	Laboratory MMAD (µm)	Concentration (µg/m^3)
30	2.2	23.7
50	1.74	7.85
60	1.74	8.80
MMAD = mass median aerodynamic diameter		

Table_Apx E-14. Guinea Pig Experimental 3-Hour/Day Exposure Concentrations Producing Tracheobronchial Surface Doses Equivalent to Workers Exposed 0.5 Hours/Day

Workplace MMAD (µm)	Laboratory MMAD (µm)	Concentration (mg/m^3)
30	3.12–3.26	2.30
50	3.12–3.26	0.59
60	3.12–3.26	0.66
MMAD = mass median aerodynamic diameter		

Table_Apx E-15. Worker Exposure Concentrations Producing Tracheobronchial Surface Doses Equivalent Dose at 6.5 Weeks in Rats Exposed to 2.2 µg/m^3 for 6 Hours/Day

Workplace MMAD (µm)	Concentration[a] (mg/m^3)	8-Hour TWA (mg/m^3)
5[b]	0.14	0.009
10[b]	0.20	0.013
30[c]	0.48	0.030
50[c]	1.85	0.116
60[c]	1.65	0.103

MMAD = mass median aerodynamic diameter; TWA = time-weighted average
[a] Thirty-minute exposure duration
[b] Spray type activities
[c] Fall-type process of reactor loading with 25 kg bags of phthalic anhydride flakes.

Table_Apx E-16. Worker Exposure Concentrations Producing Tracheobronchial Surface Doses Equivalent Those in Guinea Pigs Exposed to 0.55 mg/m^3 for 3 Hours/Day

Workplace MMAD (µm)	Concentration[a] (mg/m^3)	8-hour TWA (mg/m^3)
5[b]	0.46	0.028
10[b]	0.68	0.043
30[c]	1.59	0.099
50[c]	6.19	0.387
60[c]	5.52	0.345

MMAD, mass median aerodynamic diameter; TWA, time weighted average
[a] Thirty-minute exposure duration
[b] Spray-type activities
[c] Fall-type process of reactor loading with 25 kg bags of phthalic anhydride flakes.