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Draft Human Health and Environmental Hazard Assessment for *o*-Dichlorobenzene

Technical Support Document for the Draft Risk Evaluation

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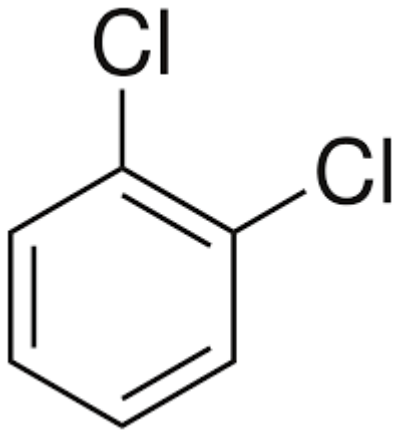

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KEY ABBREVIATIONS AND ACRONYMS

AF	Assessment factor
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMD/C(L)	Benchmark dose/concentration (lower 95% confidence limit)
BMR	Benchmark response
BrdU	Bromodeoxyuridine
BUN	Blood urea nitrogen
ChV	Chronic value
CAR	Constitutive androstane receptor
CI	Confidence interval
COC	Concentration(s) of concern
CYP	Cytochrome P450
DAF	Dosimetric adjustment factor
DCP	Dichlorophenol
DTT	Division of Translational Toxicology
EC50	Effect concentration at which 50% of test organisms exhibit an effect
EPA	Environmental Protection Agency
ETAP	EPA Transcriptomic Assessment Product
GOBP	Gene ontology biological process
GSH	Glutathione
GST	Glutathione S-transferase
HC05	Hazard concentration that is protective of 95% of the species in the SSD
HEC	Human equivalent concentration
HED	Human equivalent dose
HV	Hazard value
IRIS	Integrated Risk Information System
i.p.	Intraperitoneal
K_p	Permeability coefficient
LC50	Lethal concentration at which 50% of test organisms die
LD50	Lethal dose at which 50% of test organisms die
LO(A)EC	Lowest-observed-(adverse)-effect-concentration
LO(A)EL	Lowest-observed-(adverse)-effect-level
MIE	Molecular initiating event
MOA	Mode of action
MSigDB	Molecular Signatures Database
MTD	Maximum tolerated dose
NAMs	New Approach Methods
NIEHS	National Institute of Environmental Health Sciences
NO(A)EC	No-observed-(adverse)-effect-concentration
NO(A)EL	No-observed-(adverse)-effect-level
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPT	Office of Pollution Prevention and Toxics
OR	Odds ratio
PB	Phenobarbital
PBPK	Physiologically based pharmacokinetic
PECO	Population, exposure, comparator, and outcome

PESS	Potentially exposed and susceptible subpopulations
POD	Point of departure
(t)POD	Transcriptomic point of departure
ReCAAP	Rethinking Chronic Toxicity and Carcinogenicity Assessment for Agrochemicals Project
SD	Standard deviation; Sprague-Dawley
SSD	Species sensitivity distribution
TSCA	Toxic Substances Control Act
UF	Uncertainty factor
U.S.	United States
Web-ICE	Web-based Interspecies Correlation Estimation

SUMMARY

This technical support document (TSD) accompanies the Toxic Substances Control Act (TSCA) *Draft Risk Evaluation for o-Dichlorobenzene* (U.S. EPA, 2026i). *o*-Dichlorobenzene is an organic substance used as a solvent or additive in many products including paints and coatings, ink and toners, fuels, and air care products, among others. This draft TSD describes the use of reasonably available information to evaluate the cancer and non-cancer human health hazards. It also assesses the environmental health hazards associated with exposure to *o*-dichlorobenzene and the points of departure (PODs) to be used to estimate risks from *o*-dichlorobenzene exposures in the draft risk evaluation.

Human Health Hazard

Humans may be exposed to *o*-dichlorobenzene as a liquid or vapor (U.S. EPA, 2026c). Based on physical and chemical properties and expected exposure scenarios, the U.S. Environmental Protection Agency (EPA or the Agency) quantitatively evaluated hazards via all exposure routes. Hazards were assessed through systematic review of reasonably available evidence, which includes human epidemiology, animal toxicology, and mechanistic data (including *in vitro* studies). Previous reviews by U.S. and international government agencies were consulted for targeting the weight of scientific evidence analyses. To this end, EPA's 2006 Integrated Risk Information System (IRIS) *Draft Health Assessment of Dichlorobenzenes* and the Agency for Toxic Substances and Disease Registry's (ATSDR) 2006 *Toxicological Profile for Dichlorobenzenes* were used to identify the primary hazards and key studies. EPA independently reviewed and integrated all relevant studies identified in these assessments. These sources were supplemented by a literature search for all reasonably available information through September 2019 and an updated search through April 2025 that prioritized cancer data and screened using an updated population, exposure, comparator, and outcome (PECO) statement based on identified data gaps in the existing literature. The approach to screen the updated literature search for only cancer information was responsive to the incompleteness of the *o*-dichlorobenzene database for cancer information and consistent with U.S. EPA previous conclusion that there is there is "*inadequate information to assess carcinogenic potential"* (Section 2.4).

The non-cancer literature database was supplemented by a new study performed in collaboration with the National Institute of Environmental Health Sciences (NIEHS) Division of Translational Toxicology (DTT) (2025a) that was custom designed to address data gaps. Additionally, EPA performed a targeted search for repeated-dose studies related to respiratory toxicity as that was identified as the most important data gap. Other non-cancer outcomes were not prioritized for an updated literature search because the database was considered complete for those outcomes.

o-Dichlorobenzene is readily absorbed through the lungs and gastrointestinal tract. Evidence from read-across to other isomers with dermal absorption data and modeling estimates suggest that *o*-dichlorobenzene has measurable but limited dermal absorption, especially when accounting for volatility of the substance from unoccluded skin. *o*-Dichlorobenzene is distributed throughout the body with greater partitioning or retention in adipose tissue, kidney, liver, and bladder. Metabolism is complex, with both phase 1 and phase 2 metabolism contributing significantly to the toxicokinetic identity of the chemical. It is likely that metabolism into bioactive metabolites contributes to the toxicity of *o*-dichlorobenzene.

The database for *o*-dichlorobenzene includes studies covering both inhalation and oral exposure routes across multiple species including rats, mice, rabbits, and guinea pigs. The epidemiological database is very limited. In reviewing the reasonably available evidence, EPA concluded that the weight of scientific evidence supported dose-response analysis on four non-cancer hazard outcomes: (1) respiratory system toxicity, (2) liver toxicity, (3) kidney toxicity, and (4) body weight changes.

As noted above, and in addition to reviewing existing literature, the NIEHS DTT, at EPA's request, conducted a 5-day, whole-body inhalation toxicology and transcriptomic dose-response study to fill data gaps. The 5-day study was performed in female B6D2F1/Crl mice and female Hsd:Sprague Dawley (SD) rats exposed to *o*-dichlorobenzene or *p*-dichlorobenzene (NIEHS, 2025a). EPA independently analyzed the resulting transcriptomic data to characterize gene expression changes that can be plausibly associated with adverse toxicological outcomes based on available apical evidence (U.S. EPA, 2026l). For *o*-dichlorobenzene, which had incomplete animal data, EPA developed a novel adaptation of the *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (U.S. EPA, 2024a) that used existing mechanistic data to derive cellular process-specific transcriptomic PODs (tPODs) for mice and rats. A full description of the analyses and methods for analyzing the transcriptomic data and generating PODs can be found in EPA's *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l).

The above tPODs correspond with apical endpoints observed in the same organ systems identified in rodent toxicity studies. In selecting the best representative tPODs from each tissue, EPA was able to compare the tPODs and apical PODs to increase confidence in the values selected for use in risk estimation and to promote the use of the best available science. It was determined that the derived tPOD for liver toxicity (human equivalent concentration [HEC] = 7.48 ppm, extrapolated to a human equivalent dose of 8.26 mg/kg-day with a total uncertainty factor [UF] 30) and the derived tPOD for lung toxicity (HEC = 0.45 ppm, with a total UF 30) are a robust and representative estimates of adverse hazard thresholds for liver and respiratory toxicity, respectively. This novel approach will promote the future use and development of New Approach Methods (NAMs) in chemical risk evaluations.

The best representative apical non-cancer endpoints and PODs were derived for inhalation, oral, and dermal routes corresponding to either acute, intermediate, or chronic exposure. These PODs are associated with respiratory system toxicity, liver toxicity, kidney toxicity, and body weight changes, and all values were adjusted to human equivalent concentrations/doses (HECs/HEDs) based on continuous/daily exposure. Taken together, the apical effects observed across the database of inhalation studies whereby respiratory toxicity outcomes do not appear to accumulate greatly across duration and may decrease in severity over time, EPA is developing a single hazard value relevant for all durations of exposure to *o*-dichlorobenzene (acute, intermediate, and chronic). When substantially similar studies were not available via a different route, route-to-route extrapolation was utilized based on default exposure factors from EPA's *Exposure Factors Handbook* (U.S. EPA, 2011b). Adequate dermal toxicity studies were not available, therefore oral PODs were applied to dermal exposure with absorption differences accounted for in the exposure assessment. The most appropriate POD for inhalation exposure is nasal olfactory lesions including atrophy with an HEC of 0 ppm and total UF of 30. The HED of 11 mg/kg (UF = 30) for liver toxicity is the most appropriate POD for oral and dermal exposure.

There is insufficient evidence from cancer bioassays to evaluate the carcinogenicity of *o*-dichlorobenzene using standard approaches as described in EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005). Therefore, a weight of scientific evidence approach was employed to evaluate the extent to which the lack of inhalation carcinogenicity studies imparts significant uncertainty on the human health risk assessments for *o*-dichlorobenzene using the Rethinking Chronic Toxicity and Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) framework (Oecd, 2024; Hilton et al., 2022). Based on the weight of scientific evidence, EPA has concluded that quantitative cancer risk assessment of *o*-dichlorobenzene is not warranted and any additional cancer bioassays would be unlikely to provide evidence leading to a different conclusion. Accordingly, EPA did not identify a cancer hazard for *o*-dichlorobenzene and therefore did not derive a cancer hazard value.

Confidence ratings (Table S-1) were based on the weight of scientific evidence considering evidence integration, selection of the critical endpoint and study, relevance to exposure scenarios, dose-response considerations, and incorporation of potentially exposed and susceptible subpopulations (PESS).

Table S-1. Most Protective PODs for Each Exposure Scenario and Route for *o*-Dichlorobenzene

Target Organ System (Route)	Species	Duration Range of Co-Critical Studies [a]	Effects Observed	HEC or HED (units) [mg/m³ if Applicable]	Co-Critical PODs [a]	Reference(s)	Confidence in POD
Respiratory toxicity (inhalation)	Mice	4–90 days	Nasal olfactory tissue damage, mechanism-informed superset gene expression	BMDL = 0.45 ppm[b] (2.71 mg/m³) based on U.S. EPA (2026l); NIEHS (2025a)	0.45 ppm (UF = 30)	U.S. EPA (2026l); NIEHS (2025a)	Robust
					2.0 ppm (UF = 100)	Cho et al. (2023)	
					4.3 ppm (UF = 300)	Zissu (1995)	
Liver toxicity (oral/dermal)	Mice, rats	1–90 days	Increased liver weight, serum ALT, histopathology / mechanism-informed superset gene expression	$BMDL_{1SD}$ = 11 mg/kg-day based Umemura et al. (1996)	11.0 mg/kg-day (UF = 30)	Umemura et al. (1996)	Robust
					12.0 mg/kg-day (UF = 30)	Robinson et al. (1991)	
					8.26 mg/kg-day (UF = 30)	U.S. EPA (2026l); NIEHS (2025a)	

BMDL = benchmark dose lower confidence limit; HEC/D = human equivalent concentration/dose; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor

[a] Co-critical hazard values reflect converging evidence across studies when normalized for differences in UF. Also see Table 2-6 and Table 2-8.

[b] An 8-hour HEC will be separately calculated in the risk evaluation for use in consumer risk assessment to better align with expected exposure durations.

Environmental Hazard

EPA considered all reasonably available information identified through the systematic review process under TSCA to characterize environmental hazard endpoints for *o*-dichlorobenzene. After evaluating the reasonably available information, environmental hazard thresholds were derived for aquatic vertebrates, invertebrates, plants, and algae, as well as terrestrial vertebrates and invertebrates. These hazard thresholds are summarized in Table S-2. EPA's rationale for selecting these hazard thresholds, as well as the level of confidence based on the weight of scientific evidence, is described in Section 3 and Appendix G.

Table S-2. Environmental Hazard Thresholds for *o*-Dichlorobenzene

Receptor Group	Exposure Duration	Hazard Threshold (COC or HV)	Source
Aquatic vertebrates	Acute	0.38 mg/L	SSD
	Chronic	0.15 mg/L	Call et al. (1983) (Medium)
Aquatic invertebrates	Acute	0.38 mg/L	SSD
	Chronic	0.002 mg/L	(Tong et al., 2010) (Medium)
Aquatic plants and algae	N/A	0.22 mg/L	Galassi and Vighi (1981) (Medium)
Terrestrial vertebrates	N/A	93 ppm (559.2 mg/m^3)	Hollingsworth et al. (1958) (Medium)
Terrestrial invertebrates	N/A	184 mg/L (above water solubility limit of 141 mg/L)	ECOSAR
COC = concentration of concern; ECOSAR = Ecological Structure and Activity Relationships (ECOSAR) (predictive model); HV = hazard value; SSD = species sensitivity distribution			

Next Steps: Seeking Peer Review and Public Comment

EPA is soliciting comments from the Science Advisory Committee on Chemicals (SACC) and the public on non-cancer and cancer conclusions in this TSD. EPA is seeking SACC and public input on (1) the utilization, application, and interpretation of transcriptomic evidence to characterize *o*-dichlorobenzene hazard mechanisms and to derive a tPOD, (2) application of co-critical PODs across hazard durations, and (3) the application and conclusions of the ReCAAP analysis.

1 INTRODUCTION

This TSD presents the draft human health and environmental hazard assessment in support of the Toxic Substances Control Act (TSCA) *Draft Risk Evaluation for o-Dichlorobenzene* (U.S. EPA, 2026i), also referred to as the "draft risk evaluation," conducted for *o*-dichlorobenzene under the Frank R. Lautenberg Chemical Safety for the 21st Century Act amended TSCA on June 22, 2016. The amended law includes statutory requirements and deadlines for actions related to conducting risk evaluations of existing chemicals.

This document discusses both the human health and environmental hazard of *o*-dichlorobenzene. The risk evaluation document will summarize the results of this module and integrate the results with the exposure estimates to produce quantitative and qualitative risk estimates for all conditions of use. An outline of the subsequent sections within this assessment is provided below.

Section 2 is the human health hazard assessment. Section 2.1 introduces the approach for this assessment, including how EPA performed systematic review and the problem formulation for the analysis. Section 2.2 presents the toxicokinetics of *o*-dichlorobenzene, which influence the target organ and mechanisms of toxicity. The non-cancer assessment is provided in Section 2.3, beginning with a summary of the hazard database for critical health effects in Section 2.3.1 and 2.3.2. In addition to deriving points of departure (PODs) based on traditional apical effects observed in toxicity studies, EPA also utilized transcriptomic data to support the dose-response analysis with *in vivo* transcriptomic PODs (tPODs). The cancer hazard assessment is in Section 2.4, which applies the Rethinking Chronic Toxicity and Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) framework to evaluate the weight of scientific evidence for cancer. The human health assessment also describes how EPA considered aggregate exposures and potentially exposed and susceptible subpopulations (PESS) (Section 2.5.2). Overall conclusions for the hazard assessment, including human hazard values to be used for risk estimation, are then presented in Section 2.6. Various appendices provide additional technical details to support the above sections.

The environmental hazard assessment is Section 3. The approach and methodology are in Section 3.1. The hazard and weight of scientific evidence conclusions for aquatic species are discussed in Section 3.2 and terrestrial hazard and weight of scientific evidence conclusions are in Section 3.3. Various appendices provide additional technical details to support the above sections.

2 HUMAN HEALTH HAZARD ASSESSMENT

2.1 APPROACH AND METHODOLOGY

EPA's Office of Pollution Prevention and Toxics (OPPT) utilized systematic review processes to search, screen, evaluate, extract, and integrate reasonably available information to make conclusions about relevant adverse health effects from *o*-dichlorobenzene exposure. Following evidence integration, EPA performed dose-response analysis to derive hazard values for use in risk characterization. These values are binned into one of four duration categories, to match the corresponding human exposure scenarios for risk estimation. The durations are as follows:

- Acute (single dose or exposure to air concentration for no more than 24 consecutive hours);
- Intermediate (a repeated dosing ranging anywhere from a few days to less than 10% of lifetime, typically from short-term or subchronic studies);
- Chronic non-cancer (repeated dosing covering greater than 10% of lifetime); and
- Chronic/lifetime cancer (repeated dosing averaged over the relevant chronic period up to a full lifetime).

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.

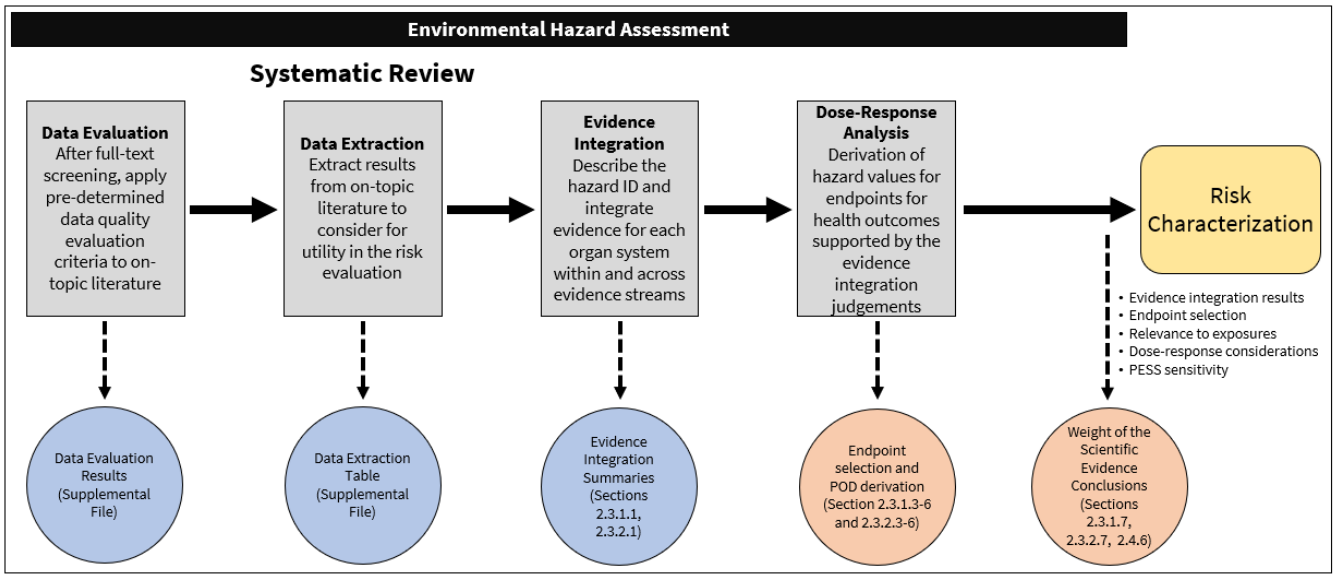

Figure 2-1. EPA Approach to Hazard Identification, Evidence Integration, and Dose-Response Analysis for *o*-Dichlorobenzene

2.1.1 Systematic Review and Focus of Analysis

The searching and screening steps of the systematic review process for *o*-dichlorobenzene 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 September 2019. An updated literature search was also performed in April 2025 with targeted criteria for refining the literature pool. The updated literature pool was refined for information related to cancer with a focus on *in vivo* data and then screened using updated Population, Exposure, Comparator, and Outcome (PECO) criteria to support EPA's cancer

classification and weight of scientific evidence analysis. The approach to screen the updated literature search for only cancer information was responsive to the incompleteness of the *o*-dichlorobenzene database for cancer information and consistent with U.S. EPA previous conclusion that there is there is *inadequate information to assess carcinogenic potential* (Section 2.4). The approach to screen the updated literature search for only cancer information was responsive to the absence of an inhalation bioassay and other data gaps in the carcinogenicity assessment (Section 2.4). The non-cancer literature database was supplemented by a new study performed in collaboration with the National Institute of Environmental Health Sciences (NIEHS) Division of Translational Toxicology (DTT) (2025a) that was custom designed to address data gaps. Additionally, EPA performed a targeted search for repeated-dose studies related to respiratory toxicity as that was identified as the most important data gap; this search identified the key inhalation toxicity study (Cho et al., 2023) that supports the inhalation POD. Other non-cancer outcomes were not prioritized for an updated literature search because the database was considered complete for those outcomes. Based on this updated search, one epidemiological study (Rodrigues et al., 2020) and one *in vitro* cell transformation assay (Lim and Seo, 2024) were identified as relevant to the cancer evaluation for *o*-dichlorobenzene. Full details and screening results for all the identified studies will be described in the *Draft Systematic Review Protocol for o-Dichlorobenzene*, to be released with the risk evaluation package (U.S. EPA, 2026k). The results of the literature searches, including screening for PECO are presented in Appendix J.

In addition to updating the literature pool for cancer outcomes, a read-across analysis was performed (Section 2.4) as part of the ReCAAP framework for evaluating *o*-dichlorobenzene carcinogenicity. The read-across analysis searched for cancer and inhalation toxicity information on structural analogs of *o*-dichlorobenzene to address the data gap from the absence of a chronic inhalation study.

In the systematic review of epidemiological studies, after screening using the PECO to define inclusion criteria, EPA adopted further filtering as a fit-for-purpose approach before studies undergo data evaluation and extraction, thereby increasing the efficiency of the systematic review. The purpose was to filter out studies that include fewer than three exposure levels. If the epidemiological studies do not have more than two exposure levels in the exposure assessment, these studies would not be used for POD derivation. As a result, the studies that were filtered out do not have study quality ratings from data evaluation and extraction. However, these studies were discussed in the hazard identification section and contribute to the overall weight of scientific evidence for their respective health effects. In addition to the further filtering, the acute exposure case reports and case series were not evaluated for data quality because they were screened as Supplemental under the PECO screening criteria described in the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021). Epidemiological studies that were filtered out and did not undergo data evaluation and extraction are listed in Appendix J.1.1. Details of the further filtering criteria and the fit-for-purpose approach are described in the *Draft Systematic Review Protocol for o-Dichlorobenzene*, to be released with the risk evaluation package (U.S. EPA, 2026k). For animal toxicology, all studies that passed PECO screening underwent data evaluation and extraction.

Previous draft and finalized governmental assessments including EPA's 2006 Integrated Risk Information System (IRIS) *Draft Health Assessment of Dichlorobenzenes* (U.S. EPA, 2006c) and the Agency for Toxic Substances and Disease Registry's (ATSDR) 2006 *Toxicological Profile for Dichlorobenzenes* (ATSDR, 2006) were used for identifying key hazard outcomes and helping summarize the hazard database. The draft IRIS assessment does not constitute an official Agency assessment, so the ATSDR assessment was given more weight when considering hazard conclusions.

Independent systematic review was performed for all studies and outcomes. This hazard assessment builds off the hazard database and conclusions from these prior assessments, updated based on current

EPA guidance, systematic review results, and in accordance with the best available science. All hazard outcomes were within the scope of the assessment but based on conclusions from these assessments, EPA identified the most well-supported hazard domains as critical health outcomes for considering endpoints to undergo dose-response analysis. These critical hazard outcomes are discussed in Section 2.3. Other hazard outcomes that are less supported as summarized in Appendix B.

Full details on all evaluated health outcomes and studies are in *Draft Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for o-Dichlorobenzene* (U.S. EPA, 2026d) and Appendix B. Overall data quality determinations and metric-specific evaluations are provided in the *Draft Data Evaluation Information for Human Health Hazard Animal Toxicology* (U.S. EPA, 2026e) and *Draft Data Evaluation Information for Human Health Hazard Epidemiology* (U.S. EPA, 2026f). Epidemiological studies that were filtered out and did not undergo data evaluation and extraction are listed in Appendix J. As described in the systematic review approach of epidemiological studies (Section 2.1.1), after PECO screening, EPA adopted further filtering to filter out epidemiological studies that include fewer than three exposure levels. The single dermal absorption study was also evaluated for data quality in *Data Quality Evaluation Information for Dermal Absorption for o-Dichlorobenzene* (U.S. EPA, 2026a).

2.1.2 Transcriptomic Data and Analysis

TSCA Section 4(h)(1)(B) requires EPA to promote the development of alternative test methods that reduce or replace vertebrate animals provided the information is "of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment . . ." [TSCA Section 4(h)(1)(B) 15 U.S.C. § 2603(h)(1)(B)]. 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 draft TSD furthers this commitment by providing mechanistic, human-relevant evidence, while advancing a rigorous, science-based transition away from traditional animal testing using a more rapid alternative method that reduces the use of animals and shortens the duration of the study. In 2023, EPA's OPPT requested NIEHS conduct 5-day, *in vivo*, inhalation studies with targeted transcriptomics for *o*-dichlorobenzene and *p*-dichlorobenzene for use in forthcoming TSCA risk evaluations (NIEHS, 2025a, b). Since that time, EPA and NIEHS have worked collaboratively to develop these data. These 5-day studies are consistent with the principles of the 3Rs of animal testing: reduce, replace, refine given that a fewer number of animals are used and the duration is much shorter. Moreover, these 5-day transcriptomic studies are a necessary bridge to validating approaches that can be used to phase out and replace traditional animal studies.

Transcriptomic-based approaches have been proposed as an alternative method to help fill data gaps for human health assessments of chemicals by providing key data on potential molecular initiating events (MIEs), mode of action (MOA), and POD estimates. Within the context of chemical risk assessment, transcriptomics is an experimental method that measures comprehensive changes in gene expression in response to chemical exposure and may provide a powerful line of evidence for relating exposure to possible adverse health effects. A full description of the methods for analyzing the transcriptomic data and generating points of departure can be found in EPA's technical report, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l). This draft document describes EPA's proposed approach for analyzing and interpreting these transcriptomic data in the draft hazard TSDs for *o*-dichlorobenzene (U.S. EPA, 2026g).

[1] Administrator Zeldin Gets EPA Back on Track to Eliminate Animal Testing After Biden Admin Halted Phase Out, https://www.epa.gov/newsreleases/administrator-zeldin-gets-epa-back-track-eliminate-animal-testing-after-biden-admin, accessed March 11, 2026

2.2 Toxicokinetics and Physiologically Based Pharmacokinetic (PBPK) Models

The below sections are primarily based on previously summarized conclusions as described in (ATSDR, 2006), supplemented by additional independent review and analysis. EPA did not identify any reasonably available studies published after 2006 that would further inform the toxicokinetics of *o*-dichlorobenzene.

2.2.1 Absorption

Oral

No data are available to evaluate oral absorption of *o*-dichlorobenzene in humans.

Animal studies suggest rapid and extensive absorption of *o*-dichlorobenzene from the gastrointestinal tract. Peak blood levels were measured 6 to 24 hours after a single gavage dose of *o*-dichlorobenzene and up to 84% of the administered dose was recovered in the urine within 72 hours (Hissink et al., 1996b).

Qualitative evidence of gastrointestinal absorption in orally dosed rats and rabbits is available from studies that measured concentrations of *o*-dichlorobenzene and/or its metabolites in blood, internal organs, or urine (Hissink et al., 1996b; Charbonneau et al., 1989; Azouz et al., 1955). Therefore, *o*-dichlorobenzene is well-absorbed from the gastrointestinal tract following oral exposures.

Inhalation

In studies of workers occupationally exposed to *o*-dichlorobenzene, metabolites were detected in urine samples (Kumagai and Matsunaga, 1997; Zenser et al., 1997; Kumagai and Matsunaga, 1995), providing qualitative evidence that *o*-dichlorobenzene is absorbed systemically following inhalation exposures.

No experimental animal studies evaluating the toxicokinetics of *o*-dichlorobenzene following inhalation exposures were identified. Qualitative evidence of respiratory absorption in experimental animals is available from studies that identified systemic toxicity following single or repeated inhalation exposures to *o*-dichlorobenzene (Hayes et al., 1985; Hollingsworth et al., 1958).

Dermal

There is limited available information concerning *o*-dichlorobenzene dermal absorption. Dermal absorption is expected to be relatively low under non-occluded conditions due to being relatively volatile (vapor pressure = 1 mm Hg (U.S. EPA, 2026c; Rsc, 2019). In Part E of the Risk Assessment Guidance for Superfund Volume I (U.S. EPA, 2004), the permeability coefficient (K_p) in cm/h for *o*-dichlorobenzene dissolved in water is estimated using an equation based on the $\log K_{ow}$ and molecular weight of a given chemical. The equation was derived based on fit to an experimental database of *in vitro* absorption data on human skin for about 90 chemicals. Based on this equation, the predicted K_p for *o*-dichlorobenzene is 4.1×10^{-02} cm/h.

There were no dermal absorption studies identified specific to *o*-dichlorobenzene. However, one study that used a unique *in vitro* system with pig skin (considered more relevant than rodents when compared to humans) inside a "cradle chamber" tested absorption of "dichlorobenzene" without identifying the isomer (Riviere et al., 2000). The specific isomer used was either *o*-dichlorobenzene, *m*-dichlorobenzene (*i.e.,* 1,3-dichlorobenzene), or a mixture, based on being in a liquid state. The dichlorobenzene was applied neat (not dissolved or diluted) within a vapor-retaining cradle chamber, in which dosing site was sealed with tape and the chamber walls enclosed the evaporating compound near the skin surface,

though the system was not fully occlusive. It is unclear if the experimental setup and amount of loading (20 µl added to a 3 cm dosing area) constitutes a finite or infinite/nondepletable dose; however, vapor concentration data indicate that DCB volatilized from the skin surface and was largely depleted by approximately 45 minutes. Back-calculation based on the limited results reported provides an estimate that 2.4% of dichlorobenzene was absorbed into the perfusate over 2 hours, with perfusate recovery plateauing by approximately 30 minutes. Any potential additional absorption from the skin depot could not be quantified. The study quality was rated "uninformative for dose-response" in *Data Quality Evaluation Information for Dermal Absorption for o-Dichlorobenzene* (U.S. EPA, 2026a) but the data can still inform the weight of scientific evidence.

In considering read-across to *p*-dichlorobenzene (1,4-dichlorobenzene), which is solid in neat form but otherwise has very similar physical-chemical properties, the available data suggests that absorption is probably similar in both isomers. The K_p for *p*-dichlorobenzene from (U.S. EPA, 2004) is 4.2×10^{-02} cm/h, almost identical to the estimate for *o*-dichlorobenzene (4.1×10^{-02}). Fractional absorption data from a test order (Charles River Laboratories, 2025) showed fractional absorption ranging from 1% to 2% for *p*-dichlorobenzene dissolved in an organic solvent and 2% to 4% for neat *p*-dichlorobenzene, comparable to the 2.4% calculated from the pig skin study (Riviere et al., 2000). See EPA's review of the Test Order study report in the *p*-dichlorobenzene public docket (Docket ID: EPA-HQ-OPPT-2018-0446) for more details on the study results and EPA's interpretation. Based on these data sources, dermal absorption estimates from *p*-dichlorobenzene will be applied to *o*-dichlorobenzene as necessary. To this end, dermal absorption modeling results from the IHSkinPerm model (accessed March 19, 2026) were also applied based on *p*-dichlorobenzene because *o*-dichlorobenzene was not available in the model dataset. The model has several parameter options and variables, but for the model of deposition over 8 h and observed for 24 h (matching the test order parameters) at the default dermal rate of 1 mg/cm^2/h for 1,000 cm^2 surface area, the fractional absorption for neat (100%) *p*-dichlorobenzene is 4.09%, basically identical to the empirical result. This further supports the validity of the test order results for use in risk assessment.

2.2.2 Distribution

In studies of the general public, *o*-dichlorobenzene has been detected in adipose tissue and breast milk (Mes et al., 1986; Jan, 1983).

In male rats exposed to [^{14}C]-*o*-dichlorobenzene via gavage, radiolabel was distributed throughout the body, with the highest levels detected in the urinary bladder, kidney, liver, and perirenal fat; the lowest levels were in brain (Hissink et al., 1996b). Bladder and kidney levels were up to an order of magnitude higher than other tissues 6 hours after treatment but decreased more rapidly. The tissue content of *o*-dichlorobenzene peaked at 6 hours post-dosing. Retention half-lives ranged from 8.7 hours for the urinary bladder to 19.3 hours for the brain. At 75 hours post-dosing, only small levels of radioactivity were detected in the tissues, mostly in the kidneys and liver. A similar gavage study in male rats showed that concentrations were greatest in adipose tissue followed by kidneys, liver, and plasma (Charbonneau et al., 1989). Distribution to urinary bladder or other tissues was not measured in this study.

In a five-day inhalation study ((NIEHS, 2025a), more details in Section 2.3.1.1.5), *o*-dichlorobenzene concentrations were measured in blood, liver, and lung. Blood concentrations were similar between mice and rats for exposures of 100 ppm or below, while mouse blood concentrations were almost 3-fold higher following 250 ppm exposures. Liver concentrations were consistently elevated in mice with a similar difference (3-fold) compared to rats at 250 ppm. Lung showed an inconsistent relative relationship, with mice showing higher concentrations at 1, 10, and 250 ppm and rat concentrations higher at 30 to 100 ppm. Overall, mice exhibited approximately 3-fold higher concentrations and

consistently higher liver concentrations than rats at 250 ppm, whereas concentrations were similar at 100 ppm and below; the reason for these interspecies differences and their relevances to humans remain uncertain.

2.2.3 Metabolism

The primary metabolic pathways for *o*-dichlorobenzene, elucidated from *in vitro* studies, rat and human liver slice studies, and *in vivo* studies in rats, start with cytochrome P450 (CYP) oxidation to form epoxides. The epoxides can undergo enzymatic cleavage, resulting in dihydrodiols (DHDs), or glutathione (GSH) conjugation. Nonenzymatic cleavage of the epoxides produces dichlorophenols (DCPs), which can be conjugated with sulfate, glucuronide, or GSH, or can undergo further oxidation to produce catechols, hydroquinones, and benzoquinones. The principle CYP involved in *o*-dichlorobenzene metabolism is CYP2E1, but minor amounts of *o*-dichlorobenzene oxidation have been detected for CYP1A1/2 and CYP3A4 (Nedelcheva et al., 1998; Hissink et al., 1996a; Bogaards et al., 1995).

The major products of *o*-dichlorobenzene metabolism include dichlorophenylmercapturic acids, 2,3- and 3,4-DCP and their GSH, sulfate, and glucuronide conjugates, 2,3-dichlorohydroquinone (DCHQ), 3,4- and 4,5d-chlorocatechol (DCC), and DHDs (including 1,2-dihydroxy-4-,5-dichloro-3,5-cyclohexadiene [1,2-DH-3,4-DCH] and 1,2-dihydroxy-3,4-dichloro-3,5-cyclohexadiene [1,2-DH-4,5-DCH]) (Hissink et al., 1996a; Hissink et al., 1996b; den Besten et al., 1992; Azouz et al., 1955).

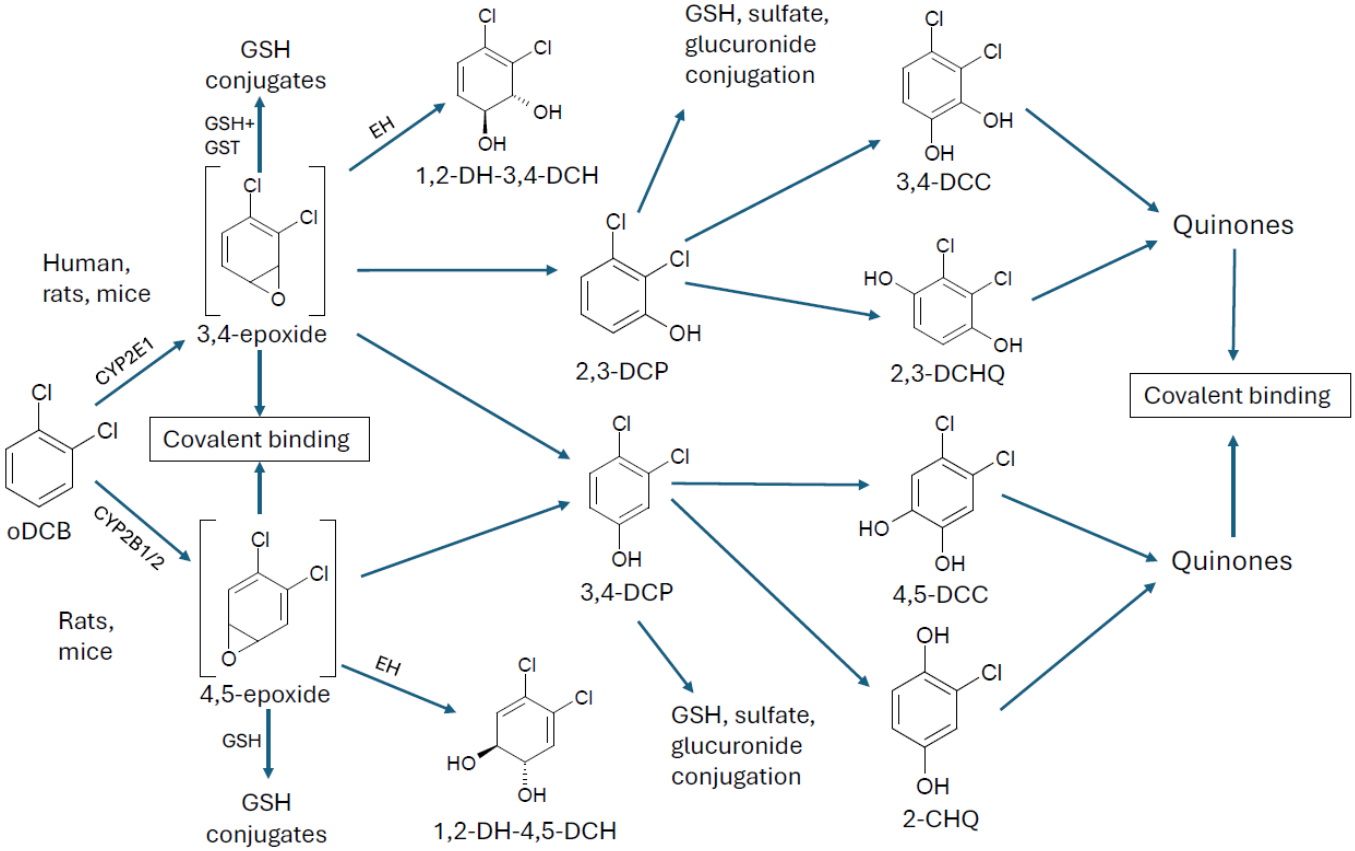

Figure 2-2. Proposed Metabolic Scheme for *o*-Dichlorobenzene

DCB = dichlorobenzene; DCP = dichlorophenol; DH-DCH = dihydroxy-dichlorocyclohexadiene; DCHQ = dichlorohydroquinone; DCC = dichlorocatechol; CHQ = chlorohydroquinone; GSH = glutathione; GST = glutathione S-transferase. Brackets indicate a postulated reactive metabolite. Adapted and updated from Muller (2002).

The reactive metabolites bind to protein and the degree of covalent binding is related to the metabolic conversion of *o*-dichlorobenzene (Nedelcheva et al., 1998; Hissink et al., 1996a). Formation of reactive metabolites or *o*-dichlorobenzene-induced toxicity was attenuated following pretreatment with CYP inhibitors (Younis et al., 2000; Valentovic et al., 1993; Fisher et al., 1991a; Stine et al., 1991) or GSH supplementation (Hissink et al., 1996a), but was enhanced by pretreatment with CYP inducers (Nedelcheva et al., 1998; Hissink et al., 1996a; Valentovic et al., 1993; den Besten et al., 1992; Stine et al., 1991; Fisher et al., 1990), GSH depletion (Stine et al., 1991), or epoxide hydrolase (EH) inhibition (Hissink et al., 1996a).

Human

CYP oxidation is a mandatory initial step in *in vitro* metabolism of *o*-dichlorobenzene by human liver microsomes (Hissink et al., 1996a). Results of *in vitro* microsomal metabolism studies indicated that CYP2E1 is the dominant human CYP isozyme responsible for *o*-dichlorobenzene metabolism, with lower amounts of oxidation catalyzed by CYP1A2, CYP1A1, and CYP3A (Nedelcheva et al., 1998; Hissink et al., 1996a; Bogaards et al., 1995). The products of *o*-dichlorobenzene metabolism by human liver microsomes include 2,3- and 3,4-DCP, DHDs, and GSH-epoxide conjugates (Hissink et al., 1996a).

The inclusion of ascorbic acid in the microsomal incubation did not significantly decrease covalent binding of *o*-dichlorobenzene metabolites to microsomal proteins (Hissink et al., 1996a), suggesting that benzoquinones do not contribute significantly to covalent binding in humans. The addition of GSH to the microsomal mixture decreased the amount of covalent binding by *o*-dichlorobenzene metabolites, while addition of an EH inhibitor decreased the amount of DHDs and increased the degree of covalent binding by *o*-dichlorobenzene metabolites (Hissink et al., 1996a). The combined addition of GSH and glutathione S-transferase (GST) to human microsomes significantly increased the amount of GSH-epoxide conjugates generated relative to that formed by the addition of GSH alone, demonstrating that most of the GSH-epoxide formation in human microsomes occurs enzymatically.

In cultured liver slice studies, the majority of *o*-dichlorobenzene metabolites consisted of GSH/cysteine conjugates for fetal human-derived tissues, with lesser amounts of glucuronide and sulphate conjugate metabolites (Fisher et al., 1991b). For adult human liver slices, sulphate conjugate metabolites were produced the least, while GSH/cysteine conjugates were the most abundant or were approximately equal to the amount of glucuronide conjugates (Fisher et al., 1995; Fisher et al., 1991b).

In occupational workers exposed to *o*-dichlorobenzene, 2,3- and 3,4-DCP, and 3,4- and 4,5-DCC, levels in urine samples correlated with *o*-dichlorobenzene air concentrations (Kumagai and Matsunaga, 1997, 1995).

Rat

In rat microsomes, CYP oxidation is a mandatory initial step in *in vitro* metabolism of *o*-dichlorobenzene (Hissink et al., 1996a). The products of *o*-dichlorobenzene metabolism by rat microsomes include 2,3- and 3,4-DCP, 2,3-D-CHQ, 3,4- and 4,5-DCC, and DHDs (Hissink et al., 1996a; den Besten et al., 1992).

CYP inducers increased the degree of metabolism of *o*-dichlorobenzene and enhanced the amount of covalent binding by *o*-dichlorobenzene metabolites (den Besten et al., 1992). Phenobarbital (PB) pretreatment of rats prior to microsome preparation and incubation with *o*-dichlorobenzene increased the amount of reactive metabolites formed, indicating an activating role for CYP2B isozymes, and increased

the relative amount of 3,4-DCP over 2,3-DCP (Hissink et al., 1996a). Addition of ascorbic acid to the microsomal incubation produced moderate decreases in covalent binding of metabolites to microsomal proteins, with minimal increases in the amounts of 2,3-DCHQ and 3,4- and 4,5-DCC detected (Hissink et al., 1996a; den Besten et al., 1992), suggesting that reactive metabolites other than quinones contribute to covalent binding. Decreases in the amount of covalent binding by *o*-dichlorobenzene metabolites were observed in rat liver microsomes following addition of GSH to the incubation mixture, while addition of an EH inhibitor decreased the amount of DHDs and increased the degree of covalent binding by *o*-dichlorobenzene metabolites (Hissink et al., 1996a). Inclusion of GSH with GST in the incubation mixture only slightly increased the formation of GSH-epoxide conjugates in rat microsomes relative to including GSH only, indicating that the majority of GSH-epoxide formed by rat microsomes occurs nonenzymatically. DNA binding of *o*-dichlorobenzene metabolites in the liver *in vivo* was not significantly affected by PB pretreatment, but *in vitro* binding of liver microsome-produced *o*-dichlorobenzene metabolites to calf thymus DNA increased following PB pretreatment (Colacci et al., 1990). Under the *in vitro* conditions, addition of a CYP inhibitor decreased the degree of DNA binding while addition of GSH to the PB pretreated microsomes increased the amount of DNA binding.

The majority of *o*-dichlorobenzene metabolites generated by cultured rat liver slices consisted of GSH/cysteine conjugates, with lesser amounts of glucuronide and sulphate conjugate metabolites (Fisher et al., 1991b). PB pretreatment of rats prior to preparation of liver slices enhanced the toxicity of *o*-dichlorobenzene (Fisher et al., 1990), whereas addition of a CYP inhibitor attenuated *o*-dichlorobenzene's toxicity (Fisher et al., 1991a), consistent with a role for CYP mediated activation.

Following oral dosing in rats, the urinary metabolites of *o*-dichlorobenzene were identified as phenylmercapturic acids, free 2,3- and 3,4-DCP and their sulphate conjugates, and GSH conjugates (Hissink et al., 1996b). The GSH conjugates and phenylmercapturic acid metabolites of *o*-dichlorobenzene were epoxide-derived. PB pretreatment in rats increased the degree of DCP sulphate conjugates with corresponding decreases in the amounts of free 2,3- and 3,4-DCP.

Urinary metabolites following intraperitoneal (i.p.) injection of rats with *o*-dichlorobenzene included 2,3- and 3,4-dichlorophenyl methyl sulfoxides and 2,3- and 3,4-dichlorophenyl methyl sulfones (Kato et al., 1988). Decreased hepatic microsomal CYP content and nicotinamide adenine dinucleotide plus hydrogen (NADH)-cytochrome b5 reductase activity and increased microsomal uridine 5'-diphospho-glucuronosyltransferase (UGT) activity were also observed following i.p. injection in rats. These effects may have consequences for subsequent *o*-dichlorobenzene exposures.

Pretreatment of Fischer 344 rats with CYP inducers or chemical agents that deplete hepatic GSH enhanced the hepatotoxicity of *o*-dichlorobenzene administered via i.p. injections, especially following CYP2E1 induction, while pretreatment with a CYP inhibitor attenuated the hepatotoxicity (Valentovic et al., 1993; Stine et al., 1991). Equivalent i.p. doses of *o*-dichlorobenzene produced higher serum alanine aminotransferase (ALT) levels in Fischer 344 rats compared to SD rats (Stine et al., 1991). The study authors postulated that this may be due to higher innate EH activity in SD rats. *o*-dichlorobenzene decreased hepatic and biliary GSH and increased biliary glutathione disulfide (GSSG) following i.p. injection of Fischer 344 and SD rats (Younis et al., 2000). Pretreatment with a CYP inhibitor prevented the increases of GSSG and decreases of GSH.

Mouse

Limited data regarding metabolism of *o*-dichlorobenzene in mice are available. Metabolism of *o*-dichlorobenzene by liver microsomes isolated from male and female mice produced soluble and covalently binding metabolites, but the production was higher in males than in females (Nedelcheva et

al., 1998). CYP2E1 induction via benzene pretreatment increased the levels of soluble metabolites in both sexes and covalently-bound metabolites in females, while CYP3A induction via pregnenolone 16α-carbonitrile (PCN) pretreatment decreased the amounts of soluble metabolites in both sexes and covalently-bound metabolites in males. Inhibition of CYP2E1 decreased the amount of soluble metabolites and covalent binding to proteins in microsomes from mice with and without benzene pretreatment. These results indicate the importance of the CYP2E1 isozyme for metabolism of *o*-dichlorobenzene in mice. PB pretreatment did not significantly affect DNA binding of *o*-dichlorobenzene metabolites in the liver *in vivo* but did increase *in vitro* binding of *o*-dichlorobenzene metabolites to calf thymus DNA following liver microsomal metabolism (Colacci et al., 1990). Addition of GSH to the PB-pretreated microsomes increased the amount of DNA binding. There is also evidence that CYP2B also contributes to metabolism of *o*-dichlorobenzene in mice (Hissink et al., 1997b; Hissink et al., 1996a; den Besten et al., 1992; Colacci et al., 1990).

Species Comparisons

Species differences have been identified in the *in vitro* metabolism of *o*-dichlorobenzene. Metabolic conversion of *o*-dichlorobenzene by human microsomes was higher than by microsomes from rats, although the degree of covalent binding was lower in humans compared to rats (Hissink et al., 1996a). Metabolism of *o*-dichlorobenzene by human microsomes results in equivalent levels of 2,3- and 3,4-DCP, while rat microsomal metabolism produces more 3,4-DCP than 2,3-DCP. These differences indicate the existence of multiple metabolic pathways involving CYP2E1 and CYP2B metabolism in rats and a dependence on CYP2E1 metabolism in humans. CYP2B metabolism of *o*-dichlorobenzene *in vitro* has also been implicated in the production of DNA bound metabolites in rats and mice and the degree of DNA binding was greater in rats than mice (Colacci et al., 1990). PB pretreatment did not appreciably alter DNA, RNA, or protein binding in rats or mice exposed to *o*-dichlorobenzene by i.p. injection (Colacci et al., 1990). GSH conjugation of epoxide metabolites occurs nonenzymatically in rats, but GSTs are required to catalyze the majority of this reaction in humans (Hissink et al., 1996a). Mouse microsomes metabolized *o*-dichlorobenzene at a rate seven times greater than rat microsomes, with a corresponding higher degree of covalent binding of metabolites in mice (Nedelcheva et al., 1998). Preincubation with benzene (CYP2E1 inducer) produced a greater increase in oxidation of *o*-dichlorobenzene of rats than in mice, leading to approximately equal *o*-dichlorobenzene metabolism between the two species (Nedelcheva et al., 1998). In cultured liver slice studies, human liver slices had faster metabolism and a higher degree of glucuronide, sulphate, and GSH/cysteine conjugation than rats (Fisher et al., 1995).

Collectively, these species differences in metabolism have important implications for understanding *o*-dichlorobenzene induced toxicity across exposure scenarios. Acute high-level exposure generates reactive metabolites, including epoxide intermediates, at rates that exceed hepatic detoxification capacity(Hissink et al., 1997c; Hissink et al., 1996a), leading to GSH depletion, covalent binding to cellular macromolecules, oxidative stress, and centrilobular hepatocellular injury (Younis et al., 2000; Hoglen et al., 1998; Stine et al., 1991). In contrast, long-term exposure can induce adaptive hepatic responses, such as increased GSH synthesis and upregulation of conjugation pathways, as described for xenobiotics generally (Hayes and Pulford, 1995). These pathways enhance detoxification and reduce the accumulation of reactive intermediates, thereby attenuating toxicity at comparable dose levels. Consistent with this, hepatic lesions observed at higher doses (250–500 mg/kg-day) in the 13-week study were not observed at 60–120 mg/kg-day in the two-year oral gavage bioassay in either rats or mice (NTP, 1985).

2.2.4 Elimination

Following occupational exposure to *o*-dichlorobenzene in humans, 2,3- and 3,4-DCP, 3,4- and 4,5-DCC, and dichlorophenylmercapturic acids have been detected in the urine (Kumagai and Matsunaga, 1997; Zenser et al., 1997; Kumagai and Matsunaga, 1995).

Following oral exposure of rats to [^{14}C]-*o*-dichlorobenzene, most of the radiolabel is excreted within 48 hours of dosing, with the majority (≥70%) of the radiolabel recovered in the urine and minor amounts (≤19%) recovered in the feces (Hissink et al., 1996b; Hissink et al., 1996c). The major urinary elimination products in rats and rabbits exposed orally to *o*-dichlorobenzene were mercapturic acid, sulfate, and glucuronide conjugates of 2,3- and 3,4-DCP (Hissink et al., 1996b; Azouz et al., 1955). After rats with cannulated bile-ducts received a single oral dose of [^{14}C]-*o*-dichlorobenzene, approximately 60% of the radiolabel was measured in the bile but less than 4% of the radiolabel was detected in the feces, indicating significant enterohepatic circulation (Hissink et al., 1996b; Hissink et al., 1996c). Characterization of the radioactive analytes detected in bile identified the GSH conjugates of *o*-dichlorobenzene metabolites as the dominant chemical species.

Consideration of PBPK Models

A PBPK model has been developed for oral exposure of rats and humans to *o*-dichlorobenzene (Hissink et al., 1997c). The model consists of four compartments connected by blood flow: 1) slowly perfused tissues (skin, muscle), 2) rapidly perfused tissues (kidneys, lung, spleen), 3) fat, and 4) liver. In this model, metabolism of *o*-dichlorobenzene was assumed to occur in the liver only. When equivalent doses of *o*-dichlorobenzene were administered to rats and humans, the models predicted that rats would have at least 6-fold higher levels of covalently bound metabolites in the liver. The models predict that humans may be more sensitive to *o*-dichlorobenzene-mediated toxicity if GSH depletion is the critical factor but that humans may be less sensitive if reactive metabolite binding is the critical factor; relative species sensitivity is therefore unclear. However, the predictive ability of the model for humans has not been established and there is not any greater confidence in use of the model compared to default EPA approaches. Additionally, the model does not include mice, rabbits, or non-oral exposure routes (*i.e.,* the source of the key endpoints for risk estimation). The PBPK model was therefore not used for deriving human health hazard values and default methods of dosimetry were applied.

2.3 Non-Cancer Hazard Assessment

Full details on all evaluated health outcomes and studies are in *Draft Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for o-Dichlorobenzene* (U.S. EPA, 2026d) and Appendix B. Overall data quality determinations and metric-specific evaluations are provided in the *Draft Data Evaluation Information for Human Health Hazard Animal Toxicology* (U.S. EPA, 2026e) and *Draft Data Evaluation Information for Human Health Hazard Epidemiology* (U.S. EPA, 2026f). Epidemiological studies that were filtered out and did not undergo data evaluation and extraction are listed in Appendix J. As described in the systematic review approach of epidemiological studies (Section 2.1.1), after PECO screening, EPA adopted further filtering to filter out epidemiological studies that include fewer than three exposure levels.

2.3.1 Inhalation Route of Exposure

The inhalation route of exposure is the major exposure pathway for humans. Two industrial hygiene human studies (Dow Chemical, 1992; Hollingsworth et al., 1958) provided air-sampling and health-outcome data on the respiratory system, kidney, and liver among workers who handled *o*-dichlorobenzene tasks. These studies are summarized in Appendix M and provide qualitative information related to effects in workers, but they do not provide information relevant for dose-response assessment. There are multiple laboratory animal studies via the inhalation route across a variety of

durations from 4 days up to 90 days, including a two-generation reproductive toxicity study and a developmental toxicity study in rats and rabbits in addition to the 5-day transcriptomic study by NIEHS. Key studies relevant to hazard characterization and identification are summarized below.

2.3.1.1 Summaries of Key Inhalation Studies for POD Derivation

2.3.1.1.1 Two-Generation Reproductive Toxicity Study (Biodynamics, 1989)

CD rats were exposed to 50, 150, or 400 ppm *o*-dichlorobenzene via whole body inhalation in a two-generation study. In the F_0 generation, 30 rats/sex/dose were exposed to *o*-dichlorobenzene for 7 days/week, 6 h/day for 10 weeks prior to mating and through mating, gestation, and lactation. Mated F_0 females were exposed from gestation days (GD) 0–19, with exposures stopped from GD20 through lactation day 4. Exposures in F_0 females resumed on lactation day 5 and continued until sacrifice upon weaning of the F_1 litters. F_0 males were exposed on the 7 days/week, 6 h/day exposure schedule until sacrifice upon weaning of the F_1 litters. The authors report that in the F_0 generation, analytical concentrations of *o*-dichlorobenzene were reported as 50 ± 3 ppm, 150 ± 5 ppm, and 397 ± 18 ppm.

Two pups/sex were randomly selected from each litter to form the F_1 generation, such that there were 30 rats/sex/dose and each litter from the F_0 generation was represented with at least 1 pup/sex. Low dose and medium dose pups not selected for the F_1 generation were sacrificed for pathological examination. Pups from the high dose group were removed from the exposure regimen and kept on basal diet for 11 weeks (*i.e.*, the F_1 pre-mating period). At the end of the 11 weeks, these rats were sacrificed for pathological examination.

Exposure to 50, 150, or 400 ppm *o*-dichlorobenzene via whole body inhalation in the F_1 generation began on postnatal day 29. The F_1 generation was exposed for 7 days/week, 6 h/day for 11 weeks prior to mating and through mating, gestation (GD 0–19), and lactation days 5–21. For the F_1 generation, the authors reported analytical *o*-dichlorobenzene concentrations of 51 ± 3 ppm, 151 ± 8 ppm, and 391 ± 25 ppm as the low, medium, and high exposure groups, respectively.

F_2 pups were sacrificed on lactation day 21 for pathological examination. All F_0 and F_1 adults were sacrificed for pathological examination, with selected organs, including reproductive organs, and tissues preserved. Organs and tissues from the control and high dose groups were examined, as were livers and kidneys for low- and mid-dose males (F_0, F_1), and the livers of low- and mid-dose females (F_0, F_1).

The authors reported no adverse effect of *o*-dichlorobenzene exposure on morbidity or mortality in either the F_0 or F_1 generations. In the F_0 generation, statistically significant lower weight gain in both sexes was reported during the pre-mating period (approximately 19% reduction in males and 11% reduction in females) in the high dose exposure group. In the F_1 generation, mean body weights at the beginning of the pre-mating period were statistically significantly lower than control in all exposure groups for both sexes. However, mean weight gain over the pre-mating period was comparable to control for most exposure conditions, except for males in the high exposure group (approximately 14% reduction). The authors concluded that reduced weight gains were an adverse effect of 391 ± 25 ppm *o*-dichlorobenzene exposure, and this effect was consistent for F_0 and F_1 males. The authors did not observe effects on reproductive indices (*e.g.*, mating, pregnancy, fertility) in either the F_0 or F_1 generation following *o*-dichlorobenzene. Reduced pup weights in the F_1 and F_2 generation (approximately 11% reduction at LD 28 for F_1, approximately 21% reduction for F_2) were consistently observed only at the highest *o*-dichlorobenzene exposure. The authors did not report adverse effects on pup survival, litter size, or sex distribution.

Effect of treatment were reported from histopathology examinations. In the F_0 generation, increased absolute and relative kidney weights in males were observed at the 150 ± 5 ppm and 397 ± 18 ppm doses. Similarly, increased mean absolute and relative liver weight was observed at 150 ± 5 ppm and 397 ± 18 ppm doses in males and females, as well as at the 50 ± 3 ppm dose in males (increases of approximately 11%, 20%, and 21% in low, medium, and high dose males; increases of approximately 9% and 19% in medium and high dose females). In the F_1 generation, increased relative kidney weights in males were observed at the 151 ± 8 ppm and 391 ± 25 ppm doses, although absolute kidney weights were comparable between control and exposure groups. Increased relative liver weight was observed at 151 ± 8 ppm and 391 ± 25 ppm doses in both sexes. The authors report that "almost all" F_0 and F_1 adult males and females in the high dose exposure group had hypertrophy of central lobular hepatocytes, and "in numerous" males and "several" females in the medium exposure group. Hepatocyte hypertrophy was not observed in either controls or low dose exposure groups. F_0 and F_1 males (nearly 100% in both generations) from the intermediate and high dose groups had kidney pathologies (*e.g.*, granular casts, intracytoplasmic granules/droplets) which increased in frequency and severity with dose. Serum biochemistry was not measured (Biodynamics, 1989).

These effects do not indicate adversity without other signs of toxicity such as serum liver enzymes or histopathology. The liver was not more closely examined in this study, because the study focused on reproductive and developmental outcomes (none of which were observed). Therefore, given the lack of liver measurements and the availability of other candidate studies, (Biodynamics, 1989) will not be used for dose-response analysis.

2.3.1.1.2 Developmental Toxicity Study in Rats and Rabbits (Hayes et al. (1985))

Hayes et al. (1985) presented inhalation toxicity data derived from two studies conducted in either F344 rats or New Zealand White rabbits examining maternal and developmental toxicity in a gestational model. In this study, F344 dams (n = 30–32/group) were exposed to 0, 100, 200, or 400 ppm *o*-dichlorobenzene via inhalation on GD 6–15 for 6 h/day. Similarly, pregnant New Zealand White rabbits (n = 28–30/group) were exposed to 0, 100, 200, or 400 ppm *o*-dichlorobenzene via inhalation on GD 6 to 18 for 6 h/day. Pregnant animals were observed daily throughout the experiment until they were sacrificed on GD 21 for rats and GD 29 for rabbits for maternal and fetal signs of toxicity.

In the rat study, maternal body weight and body weight gain were assessed throughout the exposure period. Dams were found to have significantly reduced body weight at 200 (GD 16) and 400 ppm (GD 9, 12, and 16). Body weight gain in dams was significantly reduced at all *o*-dichlorobenzene concentrations when assessed from GD 6 to 8 and GD 12 to 15, as well in the overall total GD 6 to 20 time frame (16.9–21.1% decrease). At sacrifice on GD 21, dam absolute liver weight was significantly increased at 400 ppm (5.6% increase), and relative liver weight was increased at 100 (4.7% increase) and 400 ppm (9.9% increase) (kidney weight was assessed but was not impacted by chemical exposure). Markers of reproductive toxicity in dams, including implantation sites, corpora lutea, resorptions, and sex ratio were unaffected by *o*-dichlorobenzene exposure. Fetal observations of body weight, crown-rump length, and various examined visceral and skeletal malformation markers were also unaffected by *o*-dichlorobenzene exposure, aside from delayed ossification of the lumbar vertebrae at 400 ppm (7.5% increased incidence).

In the rabbit study, maternal body weight measured daily was unaffected by exposure except for significantly reduced body weight at GD 19 and 29 in the 100 ppm group. Maternal body weight gain was significantly reduced only during the GD 6 to 8 observation period at all *o*-dichlorobenzene concentrations. Overall maternal body weight from GD 6 to 28 was significantly reduced at 100 ppm (38.9% decrease), but this effect did not follow a dose-response trend and was not observed at higher

doses. Maternal absolute and relative liver and kidney weights examined on GD 29 were not impacted in any *o*-dichlorobenzene treatment group. Markers of reproductive toxicity in pregnant rabbits, including implantation sites, corpora lutea, and resorptions, were unaffected by *o*-dichlorobenzene exposure. The ratio of male to female offspring was significantly different in the 200 ppm group, but this effect was not significantly altered in the 400 ppm group. Fetal observations of body weight, crown-rump length, and various examined visceral and skeletal malformation markers were unaffected by *o*-dichlorobenzene exposure.

Overall, the only reported adverse toxicity outcome observed in the study by Hayes et al. (1985) was reduced body weight or body weight gain observed in pregnant F344 rats and New Zealand White rabbits. The largest magnitude of a body weight response observed was reduced body weight gain from GD 6 to 8 in rats at a lowest-observed-adverse-effect-concentration (LOAEC) of 100 ppm. However, there is some uncertainty in the adversity of this effect as this reduced weight gain effect is reported over only a few days and not consistently occurring throughout observation periods. Nevertheless, reduced total maternal body weight gain in rats from GD 6 to 20 was also observed at a LOAEC of 100 ppm, but this reported timeframe effect did not follow a dose-response trend. Maternal body weight effects from Hayes et al. (1985) and considerations for dose-response modeling are further discussed among the broader hazard database in Appendix K.

2.3.1.1.3 Inhalation Study of Male Swiss OF_1 Mice (Zissu, 1995)

Zissu (Zissu, 1995) exposed male Swiss OF_1 mice (females not tested) to 64 ± 5.6 ppm or 163 ± 10.1 ppm *o*-dichlorobenzene via whole-body inhalation exposure for 6 hours/day, 5 days/week in 3 different exposure durations: (a) 4 consecutive days ("4 day exposure"), (b) 5 consecutive days in the first week, followed by 4 consecutive days the second week ("9 day exposure"), or (c) 5 consecutive days in the first week, 5 consecutive days in the second week, and 4 consecutive days in the third week ("14 day exposure"). Control mice were exposed to filtered air. At the end of the exposure period, trachea, lungs, and nasal cavity were removed for histopathological examination. The author stated, but did not show data, that no mortality was observed in the control or exposed mice. No histological or inflammatory effects were observed in control mice, although no incidence or statistical data were provided. Similarly, the author reports that the trachea and lung histopathology was no different from controls following *o*-dichlorobenzene exposure, although no incidence or statistical data were provided. Exposure to *o*-dichlorobenzene affected the olfactory epithelium at 64 ± 5.6 ppm but without providing incidence or statistical data. Severity of pathological lesions decreased with increased duration, with "very severe change" observed at the 4 day exposure, "severe change" observed at 9 day exposure, and "moderate change" after 14 days of exposure. As the severity is reported to lessen with longer exposure durations, this result suggests that mice may acclimate to the exposure. The study reports, but does not include incidence or summary statistics, a complete loss of olfactory epithelium in severe cases. This study only qualitatively reported histopathological injury and did not calculate any statistics to quantify the responses across the study population.

Without statistics or the incidence data the histopathology cannot be benchmark dose (BMD) modeled, so the 64 ± 5.6 ppm dose was established as a lowest adverse effect concentration (LOAEC) of 64 ppm. This study received a medium overall quality determination. However, the intended purpose of the study was not to obtain a point of departure; as such, EPA acknowledges uncertainty in using it to derive one.

2.3.1.1.4 90-day OECD Guideline Inhalation Study of Mice (Cho et al. (2023))

In a 90-day inhalation toxicity study in accordance with OECD test guideline 413, Cho et al. (2023) exposed 6 week old male and female B6C3F1 mice to 30 ppm, 60 ppm, or 120 ppm via whole body inhalation exposure for 6 h/day, 5 days/week for 13 weeks [Note: EPA does not have the individual

animal data, only the summary data in the Cho publication]. The authors reported chamber *o*-dichlorobenzene concentrations of 30.04 ppm (± 1.06 ppm), 59.95 ppm (± 1.97 ppm), and 122.83 (± 13.67 ppm) for the nominal concentrations of 30 ppm, 60 ppm, and 120 ppm, respectively. At the end of the 13-week exposure period, blood samples were collected and organs/tissues were examined for effects of *o*-dichlorobenzene exposure. The authors reported, but did not provide data, that no adverse clinical signs nor decreases in food consumption were noted during exposure. Body weights for males decreased at all tested doses (approximately 10%, 9%, and 5% at low, medium, and high dose, respectively), while female body weights were reduced approximately 8% at 30.04 ppm and 6% at 59.95 ppm, but not different from controls at 122.83 ppm.

Most hematological data did not demonstrate *o*-dichlorobenzene dose-related changes. The few hematological endpoints demonstrating dose-responsive changes showed decreases relative to controls for both male and female mice. Similarly, blood biochemistry parameters were largely unaffected in both males and females following *o*-dichlorobenzene exposure. A single blood biochemistry parameter in male mice demonstrated dose-related changes, showing an increase in alkaline phosphatase (ALP) with increasing *o*-dichlorobenzene exposure. Organ weights in both male and female mice were largely unaffected by *o*-dichlorobenzene exposure. Dose response decreases in absolute spleen weights were observed in males and females, as well as in the absolute brain weights of female mice. Changes in the absolute heart weights were reported for males and females, although these effects did not consistently change with increasing dose. Relative organ weights were also largely unaffected by *o*-dichlorobenzene exposure. Dose responsive decreases in relative spleen weights were reported in both male and females, with relative epididymides weights showing dose-responsive increases in males. Alterations in other relative organ weights were reported in males (lung, testes, brain) and females (brain); however, these effects did not consistently change with increasing dose.

Histopathological analysis observed mild to marked liver effects in the 122.8 ppm male mice, including mineralization, necrosis, and mononuclear cell infiltration. Liver histopathological effects in female mice were limited to mild mononuclear cell infiltration in the 122.8 ppm dose, although interpretation is limited as the control female mice showed marked mononuclear cell infiltration.

In contrast, nasal cavity histopathology was reported in both male and female mice, with female mice more sensitive than males. Nasal cavity histopathological effects in females were reported at all tested doses and included mild to marked atrophy, basil cell hyperplasia, respiratory metaplasia, eosinophilic globules and dilatation of glands. These nasal cavity histopathology, except for atrophy and dilatation of the glands, were also observed in male mice. The nasal cavity histopathology was reported at all tested concentrations, although the severity was not consistently related with exposure dose. Based on these findings, the authors conclude that *o*-dichlorobenzene inhalation exposure induced upper respiratory damage in male and female mice and hepatotoxicity in male mice (Cho et al., 2023). Cho et al. (2023) establishes a LOAEC of 30 ppm based on multiple pathologies in nasal cavity at all tested concentrations.

EPA attempted BMD modeling for eosinophilic globules in males in Cho et al. (2023) using a default benchmark response (BMR) for dichotomous outcomes of 10% extra risk. See Appendix A and *Draft Benchmark Dose Modeling Results for o-Dichlorobenzene* (U.S. EPA, 2026b) for full BMD modeling details including statistical tests, results from all models, and any associated graphs[2]. However, EPA does not have confidence in the results of this analysis because the $BMDL_{10}$ value of 0.289 ppm is

[2] All modeling was performed using EPA's BMDS online modeling suite, and PODs from animal studies were duration- and dosimetrically-adjusted to HECs based on continuous exposure prior to modeling.

almost 10-fold lower than the lowest dose tested (HEC = 2.0 ppm). Therefore, the LOAEC is a more reliable hazard value for use in risk estimation. EPA assigned a LOAEC of 30 ppm based on multiple pathologies in nasal cavity at all tested concentrations for Cho et al. (2023).

2.3.1.1.5 2025 NIEHS 5-Day Transcriptomic Study

Study Design

The 2025 5-day transcriptomic study was performed in female B6D2F1/Crl mice and female SD rats (U.S. EPA, 2026l; NIEHS, 2025a). A chronic study via inhalation is not available for *o*-dichlorobenzene, which was a major factor leading to EPA's request for NIEHS to conduct the 5-day transcriptomic study. In this study, female rats and mice were exposed for 6 hours on 5 consecutive days to 0, 1, 10, 30, 100, and 250 ppm *o*-dichlorobenzene. An additional dose group for rats was included in the study. This group was exposed to concentrations of 0, 1, 10, 30, 100, 250, and 500 ppm for 6 hours on 5 consecutive days. These concentrations were based on data in the published literature and further confirmed through a pilot study in a limited number of animals to determine suitability of exposure concentrations for the planned full study duration. Given that existing toxicology studies do not report gender specific effects, only female rodents were used for this study to reduce the overall study size. Measurement of apical endpoints and transcriptomic analyses were performed on lung, heart, liver, kidney, and ovary. Nasal tissue was not evaluated. The complete methods and data for the 5-day, *in vivo*, inhalation study with targeted transcriptomics performed by the NIEHS DTT for *o*-dichlorobenzene is available and will not be described here in full detail (NIEHS, 2025a). A table of the primary study parameters can be found in Table 2-1 and Table 2-2.

Table 2-1. Summary of Transcriptomic Study Parameters for *o*-Dichlorobenzene in Rat

Parameter	Value
Species	Rat
Strain	Hsd:Sprague Dawley (SD rats)
Sex	Female
Age	7 to 8 weeks post acclimation
Sample Size	n = 10 vehicle control; n = 5 treatment group
Route of Exposure	Whole-body inhalation
Vehicle	Preheated nitrogen
Doses	0, 1, 10, 30, 100, 250, 500 ppm
Dosing Frequency	Once per day for 6 hours
Dosing Duration	5 days
Sacrifice Time After Last Dose	24 h
Organs Evaluated	Heart, kidney, liver, lung, and ovary

Table 2-2. Summary of Transcriptomic Study Parameters for *o*-Dichlorobenzene in Mouse

Parameter	Value
Species	Mouse
Strain	B6D2F1/Crl
Sex	Female
Age	9 to 10 weeks post acclimation
Sample Size	n = 10 vehicle control; n = 5 treatment group[a]
Route of Exposure	Whole-body inhalation
Vehicle	Preheated nitrogen
Doses	0, 1, 10, 30, 100, 250 ppm
Dosing Frequency	Once per day for 6 hours
Dosing Duration	5 days
Sacrifice Time After Last Dose	24 h
Organs Evaluated	Heart, kidney, liver, lung, and ovary

[a] The 250 ppm dose group had a sample size of n = 8, with one animal euthanized moribund and excluded.

Because a review of existing toxicity data for *o*-dichlorobenzene identified adverse health outcomes in liver, lung, and kidney, gene expression analyses focused on these tissues (Mörbt et al., 2011; ATSDR, 2006; U.S. EPA, 2006c; Zissu, 1995; Hayes et al., 1985; NTP, 1985; Haskell, 1982; Hollingsworth et al., 1958).

Results of Apical Endpoints in the 5-Day Study

Alongside examining gene expression, the NIEHS study evaluated traditional apical endpoints such as clinical biochemistry and organ weights. For liver endpoints, a significant dose-response trend was identified for absolute and relative increased liver weight in both mice and rats with absolute and/or relative liver weight significantly increased at 100 ppm and above. No changes were reported for relevant clinical chemistry measurements in rats other than glucose and bile salts; bile salts demonstrated a strong dose-response trend and statistically significant increases at 250 and 500 ppm.

In mice, ALT, aspartate aminotransferase (AST), and bile salts were all statistically significantly increased in a dose-responsive manner at 250 ppm, with ALT dose-responsively increasing at 30 ppm and above. Organ weights, ALP, and cholesterol demonstrated a significant dose-response trend. Serum ALP interestingly showed a dose-responsive *decrease* compared to controls while the other metrics all increased. These apical liver measurements from the NIEHS study were BMD modeled in accordance with guidance (U.S. EPA, 2012b). All modeling was performed using EPA's BMD Software (BMDS) online modeling suite, and PODs from animal studies were duration- and dosimetrically-adjusted to HECs based on continuous exposure prior to modeling. Absolute and relative liver weights were increased at 100 ppm and above, with serum ALT dose-responsively increased above 10 ppm and becoming statistically significant at 250 ppm. Both measures demonstrated a dose-responsive trend across the dose range. A 10% relative increase in liver weight is well-established as a BMR for adverse liver toxicity from prior risk evaluations and other Agency assessments, therefore 10% relative deviation (RD) was selected as the BMR for liver weight; the default BMR of 1 SD for a continuous measurement

was also run for comparison. Serum ALP was modeled using the default 1SD BMR. Only absolute liver weight was successfully modeled with a $BMCL_{10}$ of 22 ppm; while there was an overall positive trend for the other measurements, small sample sizes and inconsistent responses below 30 ppm likely contributed to the inadequate model fits. See Appendix A and *Draft Benchmark Dose Modeling Results for o-Dichlorobenzene* (U.S. EPA, 2026b) for full BMD modeling details including statistical tests, results from all models, and any associated graphs.

For kidney effects, a significant dose-response trend was identified for relative increased kidney weight in both mice and rats with absolute and/or relative kidney weight significantly increased at 100 ppm and above in rats and at the highest dose of 250 ppm in mice. Mice did not demonstrate changes in relevant clinical chemistry measurements (blood urea nitrogen [BUN] and creatinine). In rats, creatinine measurements showed a non-significant decrease at 100 ppm and above which would not indicate adversity.

Relevant observations for respiratory effects were limited to lung weight. Relative lung weight was significantly increased in rats at the highest concentration of 500 ppm (NIEHS, 2025a). There was no significant change in mouse lung weight.

2.3.1.2 Bioinformatic and Transcriptional Dose-Response Analysis

2.3.1.2.1 Bioinformatic Analysis: Methodology

EPA performed transcriptional analyses demonstrating differential patterns of gene activation and repression across doses and species. Based on these results, EPA performed dose-response analysis on these gene responses. EPA recognizes that there are many possible approaches to evaluating transcriptomic data and that bioinformatic methodologies, particularly for use in risk assessment, remain an active area of research. EPA expects these methodologies will continue to develop and evolve over the next few years. As described below and in more detail in the draft technical report, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l), EPA has evaluated several different approaches for analyzing and interpreting these transcriptomic data which leveraged the existing apical and mechanistic data for *o*-dichlorobenzene to best inform the identification of PODs. EPA's objective with this work is to develop tPODs that will be health protective, mechanistically-derived, human-relevant, and predictive of the doses/concentrations linked to traditional toxicological adverse effects that are relevant for risk characterization.

Functional interpretation of individual gene responses aggregated into gene sets utilized several databases to help inform biological interpretation of gene expression responses including Gene Ontology Biological Process (GOBP) and the Molecular Signatures Database (MSigDB) (Consortium, 2025; Castanza et al., 2023; Liberzon et al., 2015; Subramanian et al., 2005; Ashburner et al., 2000). The stepwise approach to tPOD development conducted by EPA is detailed below:

1. **Initial differential gene expression analysis to determine whether there was sufficient evidence of chemical-induced gene disruption to perform BMD modeling and derive tPODs.**
   Briefly, multiple tissues (kidney, liver, lung, heart, and ovary) in both mice and rats exhibited sufficient dose-responsive disruption of gene expression following 5-day inhalation exposure to *o*-dichlorobenzene to allow BMD modeling and the derivation of tPODs. See Section 4 of EPA's technical support document, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l). Because a review of existing toxicity data for *o*-dichlorobenzene identified adverse

health outcomes in liver, lung, and kidney (Mörbt et al., 2011; ATSDR, 2006; U.S. EPA, 2006c; Zissu, 1995; Hayes et al., 1985; NTP, 1985; Haskell, 1982; Hollingsworth et al., 1958), only analyses in those tissues are presented.

2. **Mapping of dose-responsive genes to established, publicly available gene set collections from two public databases (GOBP and MSigDB) as well as custom-curated gene sets to summarize individual gene-level dose-response modeling into functional units.**
Mapping genes to these gene sets can assist in better interpretation of biologically meaningful responses, in contrast with individual gene-level analyses, as the coordinated changes in biological signaling at the gene set or pathway level represent a robust activity rather than potentially spurious changes. Further, as shown in the studies summarized in Section 2.3.1.1.1 through Section 2.3.1.1.5, point of contact toxicity in the respiratory tract was identified for the inhalation route of exposure as the most sensitive endpoint. Thus, the MSigDB collection of gene sets was also subset into groups of expertly curated terms, called supersets, to investigate dose-responsive changes in gene expression associated with potential mechanistic key events for *o*-dichlorobenzene, including general toxicity and cell death (Mörbt et al., 2011; ATSDR, 2006; U.S. EPA, 2006c; Zissu, 1995; Hayes et al., 1985; NTP, 1985; Haskell, 1982; Hollingsworth et al., 1958). The superset terms were categorized as follows: apoptosis, cytotoxicity, inflammation, oxidative stress, necrosis, or a combination of all five terms.

    - For respiratory toxicity, these cellular pathways are supported by *in vitro* data from (Mörbt et al., 2011), which found that apoptosis and oxidative stress response signaling were upregulated in a human lung epithelial cell line exposed to *o*-dichlorobenzene.
    - Targeted investigations suggest that liver toxicity from *o*-dichlorobenzene in rats involves reactive oxygen species (Gunawardhana et al., 1993) and is associated with activation of inflammatory signaling cascades through liver macrophage Kupffer cells (Younis et al., 2003; Hoglen et al., 1998). *o*-dichlorobenzene-induced Kupffer cell activation appears to promote lipid peroxidation and protein adduct formation which correlated with apical indicators of liver injury, with peak response around 24 hours after a single i.p. dose and decreasing thereafter (Hoglen et al., 1998).
    - Specific mechanistic data are not available for kidney, but similar mechanisms likely underly the observed apical effects. See Section 4.2 of EPA's technical support document, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l) for additional details.

3. **Derivation of tPODs for lung, liver, and kidney.**
For the three collections of gene sets (GOBP, full landscape of MSigDB, and mechanism-informed curated subset of MSigDB supersets), EPA derived tPODs for lung, liver, and kidney to correspond with the identified apical data for these organs. tPODs were derived for these tissues in both mice and rats in two distinct ways:

    - The lowest (*i.e.*, most sensitive) value of the distribution of gene sets.
    - The 5th percentile value of the distribution of gene sets.

Together, these approaches derived a total of six tPOD values that are presented below for each rodent species and tissue for *o*-dichlorobenzene.

While an additional 10 tPOD values were derived for each individual superset term (apoptosis, cytotoxicity, inflammation, oxidative stress, necrosis) using the lowest and 5th percentile value approaches, only the combined MSigDB superset tPODs are presented here. The combination of genes within any of the five superset terms relating to the plausible MOA was determined by

EPA to be most appropriate to use to get a sense of the large-scale biological impacts of *o*-dichlorobenzene exposure. This determination was informed by a recognition that individual superset terms often were too limited in scope to be informative, and that the combined set of superset terms exhibited better performance than any individual superset. Given the sensitivity of this novel approach for identifying early transcriptional signatures of toxicity, merely applying the most sensitive (*i.e.* lowest) gene set or superset (which may be an outlier, representative of transient effects, adaptive, and/or not solely driving observed toxicities) would be less reliable as an indicator of adverse response data than the 5th percentile of the combined supersets which were informed by mechanistic data. tPOD values derived from all analyses are presented in more detail in Section 4.2.3 of EPA's draft technical support document, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l). This supporting document also provides information describing why, for this analysis, the combined set of superset terms is preferred over reliance on any individual superset.

4. **Comparison of six tPOD methods with previously described transcriptomic methods and apical effects data.**
Within the six tPOD derivation approaches presented, EPA evaluated the relevance of previously described *Standard Methods for Development of EPA Transcriptomic Assessment Products* (ETAP) (U.S. EPA, 2024a) which used a highly conservative approach for deriving a tPOD (*i.e.* lowest GOBP) and was developed primarily for chemicals with no existing or repeat dose toxicity studies from which to inform mechanism or derive toxicity values using traditional risk assessment methods. In addition, the coordinated transcriptional changes used to identify the tPOD for ETAP does not necessarily discriminate between specific hazards, adverse or adaptive effects, nor is it intended to directly infer a mechanism or MOA (U.S. EPA, 2024a). On the other hand, a review of the existing data for *o*-dichlorobenzene identified adverse health outcomes in liver, lung, and kidney (Mörbt et al., 2011; ATSDR, 2006; U.S. EPA, 2006c; Zissu, 1995; Hayes et al., 1985; NTP, 1985; Haskell, 1982; Hollingsworth et al., 1958). Therefore, EPA determined that the precautionary approach utilizing the lowest value of the distribution of gene sets is not appropriate and that the mechanism-informed collection of gene sets is more representative of adverse effects for *o*-dichlorobenzene compared to the full landscape of the gene set collections. This is because *o*-dichlorobenzene has multiple high and medium quality studies which describe the apical outcomes relevant to hazard (apoptosis, necrosis, oxidative stress, cytotoxicity, and inflammation).

5. **Determination of appropriate tPOD for *o*-dichlorobenzene risk evaluation based on the performance of fit-for-purpose analyses.**
EPA is proposing the use of aggregated gene sets for tPOD derivation as they are often defined from experimental or computational studies establishing functional effects at the cellular level or, in some cases, at the animal level. Although EPA shows the 5th percentile BMDL median values using the GOBP and the full landscape of MSigDB gene sets, EPA is ultimately proposing to use the 5th percentile BMDL median values from the combined MSigDB supersets as tPODs relevant for consideration of human health hazard for *o*-dichlorobenzene since mechanistic and traditional toxicity studies are available to inform gene set selection. The novel aspect of EPA's proposed approach of using the 5th percentile of the combined MSigDB supersets for *o*-dichlorobenzene is that it used the available hazard database to anchor the gene superset selection to specific mechanistic and apical toxicity data. Therefore, the tPODs are more reflective of specific biological response processes that are changed early after *o*-dichlorobenzene exposure. While the use of the GOBP and the full landscape of MSigDB gene sets to identify a tPOD are presented here for comparative purposes, these tPODs may be more

appropriate for chemicals lacking traditional or mechanistic toxicity data as these gene set collections represent a more global, untargeted landscape of biological mechanisms and pathways.

The 5th percentile of the combined MSigDB supersets approach, in particular, derived tPOD values that were consistently within 1.5-fold of the lung and liver apical human equivalent toxicity values (after consideration of relevant uncertainty factors [UF]) while capturing more sensitive (yet not overly sensitive) gene expression changes. Further evidence of the appropriateness of the 5th percentile of the distribution of combined MSigDB supersets lies in the fact that tPODs consistently landed around the point on the accumulation plot curve where a rapid escalation of gene set activity was observed (*i.e.*, see dashed line #6 on Figure 2-3 through Figure 2-6). Additionally, using the 5th percentile of combined MSigDB supersets to define a tPOD aligns with other established EPA risk assessment approaches used in other regulatory contexts such as for deriving numerical national water quality criteria (U.S. EPA, 1985), the Threshold of Toxicological Concern (Kroes et al., 2005), and Species Sensitivity Distribution (SSD) analysis (Dhond and Barron, 2022; Newman et al., 2000). Further, within i*n vitro* NAMs, the lower 5th percentile of the distribution of BMD values has been used to identify PODs based on preliminary chemical bioactivity screening (Friedman et al., 2020) and, more broadly, in the field of toxicogenomic research using the distribution of BMDs of gene sets in multiple *in vitro* high-throughput transcriptomic analyses (Bundy et al., 2024; Harrill et al., 2024; Harrill et al., 2021). For additional discussion of these data sets, also see *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l).

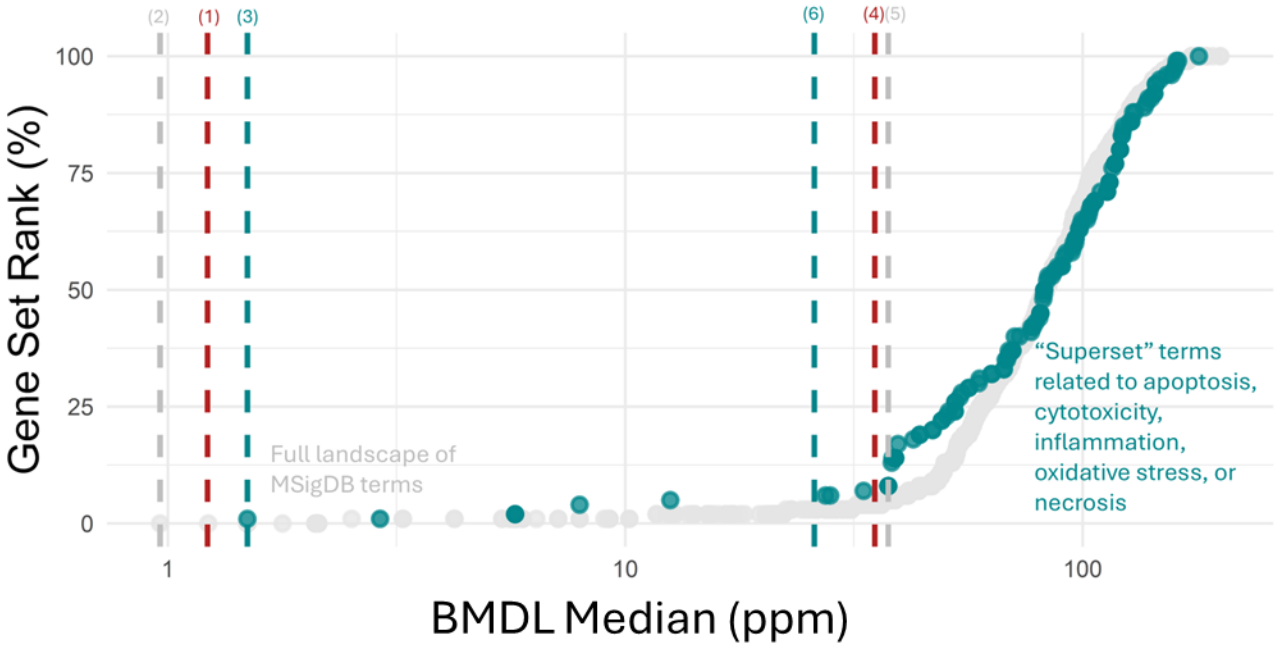

Figure 2-3. Comparison of Six Analysis Methods used to Derive a tPOD for *o-D*ichlorobenzene in Mouse Liver

The six methods are 1) the lowest (*e.g.*, most sensitive) GOBP [red], 2) lowest MSigDB [gray], 3) lowest MSigDB combined superset [cyan], 4) 5th percentile GOBP [red], 5) 5th percentile MSigDB [gray], 6) 5th percentile MSigDB combined superset [cyan].

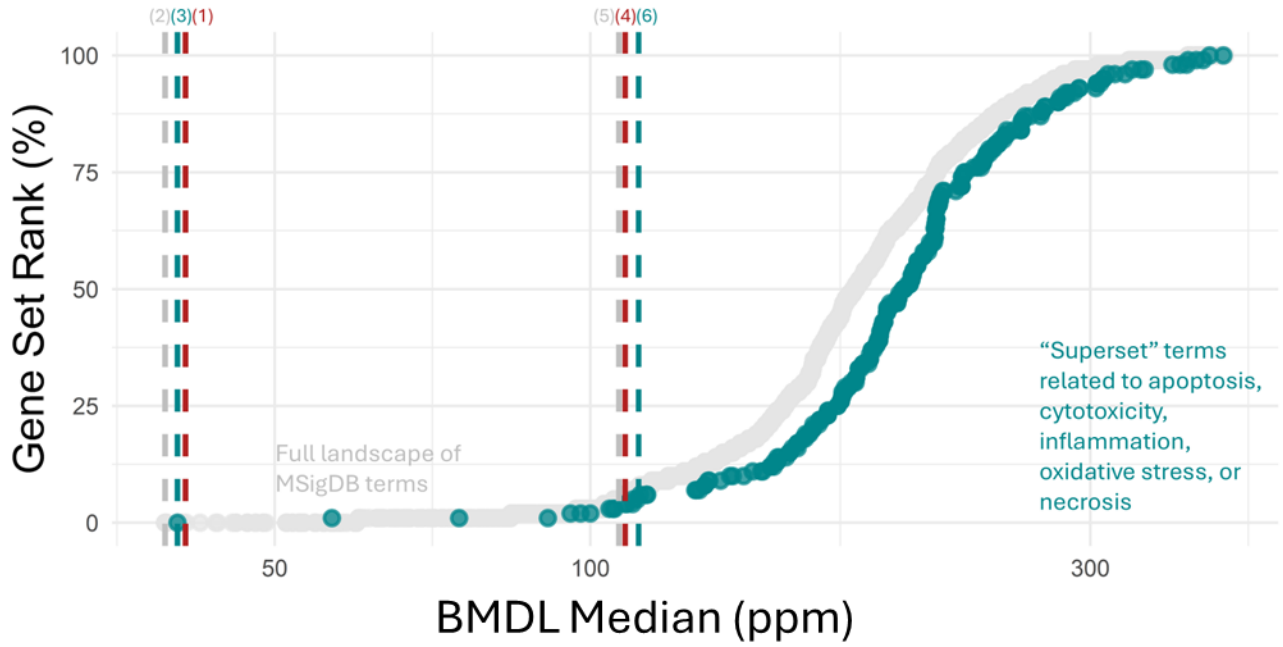

Figure 2-4. Comparison of Six Analysis Methods used to Derive a tPOD for *o*-Dichlorobenzene in Rat Liver

The six methods are 1) the lowest (*e.g.*, most sensitive) GOBP [red], 2) lowest MSigDB [gray], 3) lowest MSigDB combined superset [cyan], 4) 5th percentile GOBP [red], 5) 5th percentile MSigDB [gray], 6) 5th percentile MSigDB combined superset [cyan].

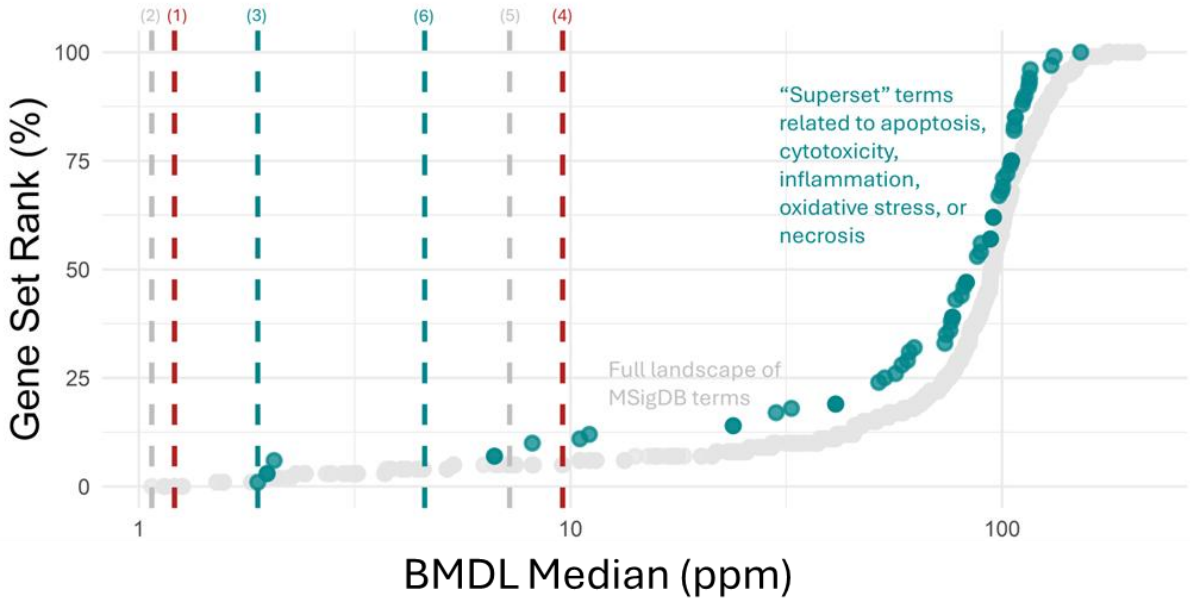

Figure 2-5. Comparison of Six Analysis Methods used to Derive a tPOD for *o*-Dichlorobenzene in Mouse Lung

The six methods are 1) the lowest (*e.g.*, most sensitive) GOBP [red], 2) lowest MSigDB [gray], 3) lowest MSigDB combined superset [cyan], 4) 5th percentile GOBP [red], 5) 5th percentile MSigDB [gray], 6) 5th percentile MSigDB combined superset [cyan].

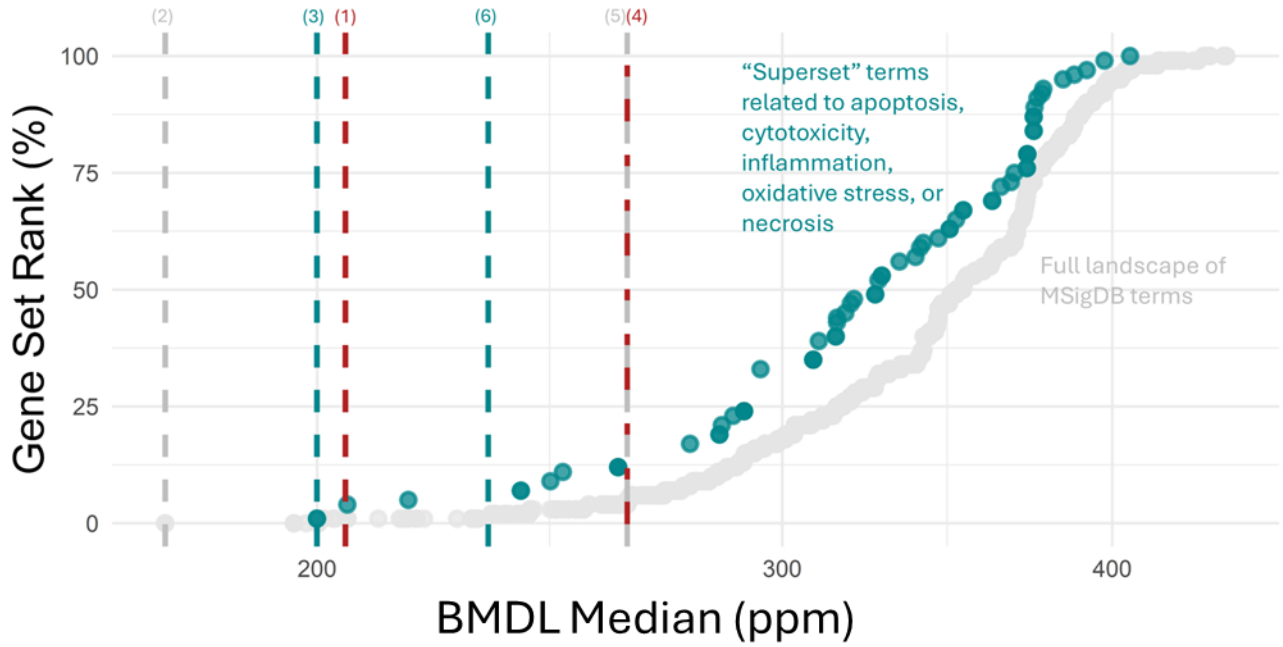

Figure 2-6. Comparison of Six Analysis Methods used to Derive a tPOD for *o*-Dichlorobenzene in Rat Lung

The six methods are 1) the lowest (*e.g.*, most sensitive) GOBP [red], 2) lowest MSigDB [gray], 3) lowest MSigDB combined superset [cyan], 4) 5th percentile GOBP [red], 5) 5th percentile MSigDB [gray], 6) 5th percentile MSigDB combined superset [cyan].

2.3.1.2.2 Bioinformatic Analysis: Results

Strikingly, across all gene set collections, the lowest terms within each species and tissue were within 1.6-fold of each other and the 5th percentile terms were within 1.2-fold of each other, demonstrating high concordance regardless of the method selected. This result helps to build confidence that the specific gene set database selected does not dramatically modify the tPOD, and that the MSigDB superset terms are representative of the full distribution of gene set terms across the MSigDB landscape.

Liver: Using the six methods to derive tPODs described above, the resultant values for mouse and rat liver can be found in Table 2-3.

Table 2-3. *o*-Dichlorobenzene tPODs Derived Using Six Methods for Mouse and Rat Liver

Chemical	Tissue	Species	Lowest GOBP tPOD	Lowest MSigDB tPOD	Lowest MSigDB Combined Superset tPOD	5th Percentile GOBP tPOD	5th Percentile MSigDB tPOD	5th Percentile MSigDB Combined Superset tPOD
o-Dichlorobenzene (CASRN 95-50-1)	Liver	Mouse	1.22 ppm	0.96 ppm	1.49 ppm	35.12 ppm	37.58 ppm	29.87 ppm
		Rat	41.10 ppm	39.29 ppm	40.38 ppm	107.95 ppm	106.48 ppm	112.49 ppm

Lung: Using the six methods to derive tPODs described above, the resultant values for mouse and rat lung can be found in Table 2-4.

Table 2-4. *o*-Dichlorobenzene tPODs Derived Using Six Methods for Mouse and Rat Lung

Chemical	Tissue	Species	Lowest GOBP tPOD	Lowest MSigDB tPOD	Lowest MSigDB Combined Superset tPOD	5th Percentile GOBP tPOD	5th Percentile MSigDB tPOD	5th Percentile MSigDB Combined Superset tPOD
o-Dichlorobenzene (CASRN 95-50-1)	Lung	Mouse	1.21 ppm	1.07 ppm	1.98 ppm	9.59 ppm	7.23 ppm	6.67 ppm
		Rat	205.00 ppm	175.20 ppm	200.00 ppm	262.08 ppm	262.08 ppm	218.79 ppm

Kidney: The NIEHS noted that 24.3% (9/37) of all mouse kidney samples for *o*-dichlorobenzene had evidence of tissue contamination, with a subset of samples identified via distinct principal component analysis clustering as potential outliers. This separation was driven by very high expression of pancreas-specific genes compared to other kidney samples which suggested possible contamination with pancreatic tissue during sample collection. These contaminated samples were excluded from the analysis, resulting in poor statistical power and modeling results for the differential expression and BMD for mouse kidney (U.S. EPA, 2026l). Furthermore, given that the mice tPODs appeared to be more sensitive than rats for both liver and lung across all tPOD derivation approaches and the strongest apical effect for kidney effects was observed in mice (NTP, 1985), EPA did not consider the rat tPODs as appropriate to use in this case where corresponding mouse data were unavailable. For these reasons, the kidney tPODs were not utilized in the draft risk evaluation. A full description of the rat kidney results can be found in Section 4.2.3 of the draft technical support document, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l).

Table 2-5. Proposed tPODs from NIEHS 5-Day Inhalation Study

Tissue (Effect)	Species	Transcriptomic POD (tPOD)	Reference
Liver (Mechanism-Informed Superset Gene Expression)	Mouse	29.87 ppm	U.S. EPA (2026l); NIEHS (2025a)
Lung (Mechanism-Informed Superset Gene Expression)	Mouse	6.67 ppm	U.S. EPA (2026l); NIEHS (2025a)
Liver (Mechanism-Informed Superset Gene Expression)	Rat	112.49 ppm	U.S. EPA (2026l); NIEHS (2025a)
Lung (Mechanism-Informed Superset Gene Expression)	Rat	218.79 ppm	U.S. EPA (2026l); NIEHS (2025a)

In considering rat and mouse responses relative to apical observations in toxicity studies, most relevant adverse outcomes were observed in mice. For respiratory toxicity, both relevant studies (Cho et al., 2023; Zissu, 1995) were in mice. For liver, the oral acute toxicity study with apical findings following a single day of exposure was also in mice (Umemura et al., 1996). Therefore, the tPODs from mice were considered most appropriate for POD selection.

2.3.1.3 Inhalation Study Database Conclusions

There are multiple laboratory animal studies via the inhalation route across a variety of durations from 4 days up to 90 days, including a two-generation reproductive toxicity study and a developmental toxicity study in rats and rabbits. In addition, there is a 5-day transcriptomic study by NIEHS. A chronic study via inhalation is not available for *o*-dichlorobenzene, which was a major factor leading to EPA's request for NIEHS to conduct the 5-day transcriptomic study. With the availability of the 5-day transcriptomic study, EPA does not consider the lack of chronic testing to be a deficiency in the overall database. As such, the database of toxicology studies for *o*-dichlorobenzene is sufficient and robust for developing non-cancer PODs for risk characterization via the inhalation route, the major route of exposure.

There are two repeat exposure inhalation studies in mice evaluating effects in the respiratory tract to *o*-dichlorobenzene, Zissu (1995) and Cho et al. (2023). Zissu scored a low for overall data quality determination because results were reported only qualitatively without any statistics compared to controls. Cho et al scored a high for overall data quality determination. Neither study showed adverse effects in the lung histopathology. Both studies show adverse effects in all tested concentrations in nasal lesions and showed LOAECs of 64 ppm and 30 ppm, respectively. Neither study established a no-observed-adverse-effect-concentration (NOAEC) and could not be reliably BMD modeled.

The 5-day transcriptomic study (NIEHS, 2025a) reported some limited apical outcomes, particularly in the liver at the higher concentrations, but the tPODs for lung and liver from mice (6.67 and 29.87 ppm, respectively; see Table 2-5) were lower than LOAECs based on apical observations in (Zissu, 1995) and (Cho et al., 2023). Due to contamination issues with the kidney samples, the kidney results are not considered reliable and therefore were not considered further in the risk evaluation. Respiratory toxicity is the most well-supported and clearly adverse outcome among the database of apical inhalation studies. Therefore, the lung tPOD is the most relevant tPOD for risk characterization of inhalation exposures and is considered further below alongside apical respiratory toxicity findings. The liver tPOD will not be considered further for the inhalation route but will be considered in Section 2.3.2 for oral POD derivation via route-to-route extrapolation along with other systemic effects.

With regards to exposure during pregnancy and pre- and post-natal exposures, there were no reported adverse effects on reproductive performance, fertility, or offspring in either generation in the two-generation reproduction study and no adverse reproductive and developmental outcomes following gestational exposure of pregnant rats and rabbits. Reduced maternal body weight was observed at all concentrations tested (100 ppm and above) (Hayes et al., 1985), however there is less consistent evidence for effects on body weight and this effect is considered supplemental to the more well-supported outcomes (see Appendix K for detailed analysis of this endpoint). Given the combined results of Zissu, Cho, and the NIEHS 5-day transcriptomic study, *o*-dichlorobenzene exposure to rodents via inhalation results in primarily point of contact effects in the respiratory system. As such, for inhalation exposure, the studies evaluating effects on the respiratory system will be protective during pregnancy and early lifestages. EPA is focusing the remainder of the POD determination for the inhalation route analysis on Zissu, Cho, and the lung tPOD from the NIEHS 5-day transcriptomic study.

In the following section, LOAECs from Zissu and Cho and the BMDL derived for the lung tPOD are converted to Human Equivalent Concentrations (HECs). In Section 2.3.1.5, EPA considers the appropriate duration for evaluating *o*-dichlorobenzene. The strengths and uncertainties of Zissu, Cho, and the NIEHS 5-day transcriptomic study with regard to selecting a POD for risk characterization are described in Section 2.3.1.7.

2.3.1.4 Dosimetric and Unit Adjustments for Inhalation Hazard Values

Dosimetric Adjustments

In the absence of a validated PBPK model or other chemical specific data-derived extrapolation factors (U.S. EPA, 2014), EPA follows established guidance for dosimetric adjustments across species (U.S. EPA, 2012a, 1994). For portal of entry effects in the respiratory region, delivered dose is based on the relative differences in the ratio of breathing rate (otherwise called ventilation rate) to surface area of a given region (known as the regional gas dose ratio, RGDR). The only portal of entry effect selected for dose-response modeling is nasal histopathology, from (Cho et al., 2023) and (Zissu, 1995) which is considered the extrathoracic region.

For the lung, EPA applied the extrathoracic region for dosimetry.

The RGDR is calculated for this effect using the following equation (using parameters for mice):

Equation 2-1. RGDR Adjustment for Extrathoracic Portal of Entry Effects

$$RGDR_{ET} = \frac{(V_E/SA_m)_A}{(V_E/SA_m)_H} = 0.27$$

Where:

$V_{E\text{-}A}$ = Daily breathing rate of mice (0.059 m^3/day, from (U.S. EPA, 1994))
$V_{E\text{-}H}$ = Updated daily breathing rate of humans (14.7 m^3/day, from (U.S. EPA, 2011a))
$SA_{m\text{-}A}$ = Mouse extrathoracic surface area (3 cm^2, from (U.S. EPA, 1994))
$SA_{m\text{-}H}$ = Human extrathoracic surface area (200 cm^2, from (U.S. EPA, 1994))

The RGDR value of 0.27 was applied to the duration-adjusted air concentration from (Cho et al., 2023) and (Zissu, 1995) to obtain the HEC.

Duration Adjustments

The studies selected for dose-response assessment utilized differing exposure durations and frequencies. In order to better compare results across studies and exposure scenarios, administered concentrations from Zissu, Cho, and the 5-day transcriptomics studies were linearly adjusted to continuous exposure (24 h/day, 7 days/week) prior to POD derivation based on Haber's Law (Haber, 1924) and in accordance with (U.S. EPA, 1994) using the following equation:

Equation 2-2. Adjusting Average Exposure Concentration or Inhalation POD for Differences in Days and Hours of Exposure Across Scenarios

$$Concentration_{continuous} = Concentration_{study} \times (\frac{D_S}{7}) \times (\frac{H_S}{24})$$

Where:

$Concentration_{continuous}$ = Adjusted air concentration/inhalation POD
$Concentration_{study}$ = Air concentration/inhalation POD from study dataset
D_s = Days per week/year exposure in study dataset
H_s = Hours per day exposure in study dataset

Unit Conversion

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 inhalation hazard values in both units. The following equation presents the conversion of the HEC from mg/m^3 to ppm.

Equation 2-3. Converting ppm to mg/m^3

$$HEC\ (mg/m^3) = HEC\ (ppm) \times \left(\frac{Molecular\ Weight}{24.45\ L}\right)$$

Where:

HEC	=	The human equivalent concentration in units of mg/m^3 or ppm
Molecular Weight	=	The molecular weight of o-dichlorobenzene (147.00 g/mol)
24.45	=	Based on the ideal gas law, 1 mole of gas occupies 24.45 L[3]

2.3.1.4.1 HEC Derivation for Inhalation PODs

- The calculated HEC LOAEC from (Cho et al., 2023) is 2.0 ppm based on the LOAEC of 30 ppm. The HEC is derived by first duration-adjusting from 6 h/day to 24 h/day (no adjustment for days/week is applied because this POD is relevant to acute exposures). The 24 h value is then adjusted to an HEC using the RGDR value of 0.27 for dosimetric differences across species based on the extrathoracic region.
- The calculated HEC LOAEC from (Zissu, 1995) is 4.3 ppm based on the LOAEC of 64 ppm. This value is adjusted in the same manner as (Cho et al., 2023). The LOAEC was adjusted for a 24h exposure from the study duration of 6 h, and the RGDR of 0.27 was then applied.
- The lung tPOD of 6.67 ppm is adjusted to an HEC of 0.45 ppm. The same adjustments were applied as stated for the two apical PODs above.

2.3.1.5 Consideration of Exposure Duration(s) Relevant for Risk Characterization

The three studies under consideration for POD derivation via the inhalation routes are derived from different durations of exposure. In the transcriptomic study, mice and rats were exposed for 5 days whereas in the Cho study mice were exposed for 90-days. The Zissu study evaluated three different durations: 1) 4 days; 2) 5 consecutive days in the first week, followed by 4 consecutive days the second week ("9 day exposure"), and 3) 5 consecutive days in the first week, 5 consecutive days in the second week, and 4 consecutive days in the third week ("14 day exposure").

Although the dose-response information provided in Zissu is primarily qualitative, this study is informative in considering the most relevant duration of exposure for characterizing risk to *o*-dichlorobenzene via the inhalation route. Specifically, severity of pathological lesions decreased with increased duration, with "very severe change" observed at the 4 day exposure, "severe change" observed at 9 day exposure, and "moderate change" after 14 days of exposure. As the severity is reported to lessen with longer exposure durations, these findings suggest that mice may acclimate to the exposure.

The findings in the Cho study at 90 days of exposure provide additional support for a hypothesis that longer durations of inhalation exposure does not lead to accumulating toxicity. Notably, after 90-days of exposure at a similar concentration of 60 ppm, severity of nasal atrophy was graded minimal to mild, compared to the severity observed in Zissu at shorter durations.

As described in Section 2.2.3, the metabolism profile provides additional evidence for the unique, duration dependent differences in *o*-dichlorobenzene toxicological profile. Acute high-level exposure generates reactive metabolites, including epoxide intermediates, at rates that exceed hepatic detoxification capacity (Hissink et al., 1997c; Hissink et al., 1996a), leading to GSH depletion, covalent

[3] The Ideal Gas Law can be used to convert between ppm and mg/m^3. At standard temperature and pressure (STP; 25°C and 760 mm Hg), 1 mole of gas occupies 24.45 L. However, when conditions differ from STP, a different gas conversion factor can be calculated using the reported experimental temperature or pressure.

binding to cellular macromolecules, oxidative stress, and centrilobular hepatocellular injury (Younis et al., 2000; Hoglen et al., 1998; Stine et al., 1991). In contrast, long-term exposure can induce adaptive hepatic responses, such as increased GSH synthesis and upregulation of conjugation pathways, as described for xenobiotics generally (Hayes and Pulford, 1995). These pathways enhance detoxification and reduce the accumulation of reactive intermediates, thereby attenuating toxicity at comparable dose levels, consistent with the absence of hepatotoxicity findings in the NTP two-year oral gavage bioassay at 60–120 mg/kg-day in both rats and mice (NTP, 1985).

Taken together, the apical effects observed in the Zissu and Cho studies combined with the metabolic profile support a preliminary conclusion that repeated exposure to *o*-dichlorobenzene via the inhalation does not lead to accumulation of effects. As such, EPA is developing a single hazard value relevant for all durations of exposure to *o*-dichlorobenzene (acute, intermediate, and chronic).

2.3.1.6 Hazard Value Derivation Applicable to Inhalation Route of Exposure

As described in Section 2.3.1.5, EPA is developing a single hazard value relevant for all durations of inhalation exposure to *o*-dichlorobenzene (acute, intermediate, and chronic). Table 2-6 provides the summary of the PODs, HECs, and proposed UFs derived from the 5-day NIEHS transcriptomic study, the Zissu study and Cho study.

Table 2-6. Candidate Respiratory Toxicity Endpoints

Study	U.S. EPA (2026l); NIEHS (2025a)	Zissu (1995)	Cho et al. (2023)
Route	*Inhalation*		
Endpoint/ Effects	Mechanism-informed superset gene expression	Nasal olfactory lesions	Nasal olfactory lesions
Duration	5 days	4 days (also 9 and 14 days)	90 days
Species	Mice	Mice	Mice
LOAEL	BMDL of 6.67 ppm	64 ppm	30 ppm
NOAEL		None	None
HEC	0.45 ppm	4.3 ppm	2 ppm
UFs	$UF_A = 3$ $UF_H = 10$ *Total UF = 30*	$UF_A = 3$ $UF_H = 10$ $UF_L = 10$ *Total UF = 300*	$UF_A = 3$ $UF_H = 10$ $UF_L = 3$ *Total UF = 100*
Proposed POD/UF	0.45 ppm	0.43 ppm	0.67 ppm

1. **Interspecies Uncertainty Factor (UF_A) of 3**
   The UF_A has a toxicokinetics and toxicodynamics component, typically each comprising 3× of the 10× UF_A. As described in Section 2.3.1.4, standard dosimetric approaches to account for toxicokinetic differences based on EPA guidance were applied to derive HECs. Therefore, only the toxicodynamic component of the UF_A is unaccounted for in the POD value and the remaining UF_A is 3.

2. **Intraspecies Uncertainty Factor (UF_H) of 10**
   EPA uses a default 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*-dichlorobenzene. There is no validated PBPK model available that can quantify human variability in toxicokinetics or toxicodynamics, so the full 10× factor applies to account for both components.

3. **LOAEL-to-NOAEL Uncertainty Factor (UF_L) of 3 or 10**
The apical HECs used to derived hazard values for Zissu and Cho studies represent a lowest-observed-adverse-effect-level (LOAEL), because an adverse effect was observed at the lowest dose. An UF_L can be either 10 or 3 depending on the dose-response, severity, and other considerations. As discussed in Section 2.3.1.1.3, the selected LOAEC for nasal toxicity from Zissu is severe and thus a 10× is applied. Cho et al. (2023) reported reduced severity of tissue damage than Zissu (1995). Thus, a 3× is applied for Cho et al. (2023). A UF_L is not applied to the tPOD because it was BMD modeled which identifies the threshold for no effect (analogous to a no-observed-adverse-effect-level [NOAEL]).

Despite differences in study design and measured outcome, the three studies reflect converging evidence normalized for differences in UFs (Table 2-6). Therefore, all three studies are considered co-critical. EPA is preliminarily determining that the inhalation hazard value for *o*-dichlorobenzene is 0.45 ppm based on effects observed in the respiratory system. The 0.45 ppm lung tPOD is selected for risk estimation over the values of 0.43 and 0.67 ppm from Zissu (1995) and Cho et al. (2023), respectively, as the 0.45 ppm value is derived from a BMDL and considering the weaknesses of the Zissu and Cho studies, EPA has more confidence in the strength of this approach. BMD modeling is a more refined and robust approach in comparison to the identification of NOAEC/LOAECs. Furthermore, the Zissu and Cho studies only provide freestanding LOAECs and therefore required the application of UF_L due to the inability to characterize doses that do not drive adversity, therefore increasing the overall uncertainty in the precision of the hazard value.

2.3.1.7 Weight of Scientific Evidence Evaluation for the Inhalation PODs

EPA has preliminarily determined that the inhalation HEC for *o*-dichlorobenzene of 0.45 ppm with a total UF of 30 will be used as a hazard value for inhalation exposures of all durations based on respiratory effects observed in the respiratory system derived from three co-critical studies, Zissu, Cho and the 5-day transcriptomic study. EPA has robust confidence in this value based on the following (also see Appendix D):

- EPA has evaluated the database of inhalation toxicology studies and identified a variety of hazard effects across durations and species. Point of contact toxicity to the respiratory tract is consistently more sensitive than systemic effects, including those during pregnancy and pre- and post-natal exposure. Point of contact toxicity to the nose and lung is protective of human health.
- In the initial 2019 systematic review, EPA identified a lack of chronic duration inhalation toxicity studies for *o*-dichlorobenzene and has collaborated with NIEHS to develop 5-day transcriptomic data. Because of multiple studies demonstrating correlation between transcriptomic results from short-term exposures to other compounds with PODs from chronic studies, EPA believes the new 5-day transcriptomic study for *o*-dichlorobenzene satisfies this database deficiency (Chang et al., 2024).
- EPA developed a novel bioinformatic analysis using existing knowledge of *o*-dichlorobenzene toxicity to develop tPODs for liver and lung tissues from rats and mice. The studies resulting in production of lung tPODs are considered among the relevant studies for deriving the hazard value.
- The Zissu and Cho studies provide LOAEC values for nasal histopathology in mice which are also considered co-critical. When deriving a risk threshold by incorporating UF differences, the 0.015 ppm value from the lung tPOD is selected over the values of 0.014 and 0.02 ppm as this

value is derived from a BMDL. In contrast, Zissu and Cho require additional UFs because a NOAEC was not established. BMD modeling results in a more statistically refined POD than using NOAEC/LOAECs, and uncertainty is reduced through the reduction of UFs.

2.3.2 Oral Route of Exposure

2.3.2.1 Summaries Of Key Oral Studies for POD Derivation

For purposes of risk characterization, oral exposure scenarios to *o*-dichlorobenzene include general population exposure to surface and drinking water. There are no available toxicity studies via the dermal route of exposure although it is anticipated that workers may have dermal exposure to *o*-dichlorobenzene during specific tasks. Thus, oral endpoint(s) are needed for route-to-route extrapolation to the dermal route. The inhalation PODs derived from Section 2.3.1 are not appropriate for route-to-route extrapolation as they are derived from point of contact toxicities in the nose and lung. There are multiple laboratory animal studies via the oral route across a variety of durations from 1 day up to chronic exposure. In addition, EPA has considered route-to-route extrapolation from systemic liver effects observed in inhalation studies as part of the dose-response evaluation for oral exposure; these include the two-generation reproductive toxicity study (Biodynamics, 1989) and the 5-day transcriptomic study by NIEHS (NIEHS, 2025a). Key studies relevant to hazard characterization and identification are summarized below.

2.3.2.1.1 Acute Studies [(Haskell, 1982) and (Umemura et al., 1996)]

In one acute study evaluating lethality at an oral dose of 1,500 mg/kg, liver necrosis in rats (Haskell, 1982) was observed. In an acute oral screening study that used only one rat per dose in a series ranging from 6 mg/kg to 1,784 mg/kg, two serum biochemical markers of liver damage, serum ALT and serum AST, were increased by at least 100% at the 172 mg/kg dose level or above (Allis et al., 1992). Liver necrosis was also first seen at that dose, while degeneration was observed at 98 mg/kg. These results are corroborated in another acute study on 5 mice per dose group that reported increased ALT two days following a single oral dose of 200 mg/kg, with necrosis to an average of 30% of the observed area (not statistically significant due to large variance) seen at 300 mg/kg (Umemura et al., 1996). No statistically significant increase was observed at 120 mg/kg. In a time-course within that study, ALT levels were statistically different from controls from 1 through 3 days following exposure.

Umemara et al. (1996) exposed groups of 5 male B6C3F1 mice to 120 mg/kg, 200 mg/kg, or 300 mg/kg of *o*-dichlorobenzene dissolved in corn oil as a single dose via intragastric administration. Control mice were administered corn oil at the same volume in the high-dose group. Two days after exposure, blood was collected for serum biochemistry and liver slices were taken for histology and immunohistochemistry. In the same study, Umemara et al. (1996) gave groups of 5 mice a single intragastric administration of 300 mg/kg *o*-dichlorobenzene in a time-course experimental design, then euthanized animals 1, 2, 3, 4, or 7 days post-exposure and collected blood and liver slices for histology and immunohistochemistry. Increased cell proliferation, measured by bromodeoxyuridine (BrdU) labeling, was observed after acute *o*-dichlorobenzene concentration, but only at the 300 mg/kg dose. Serum ALT activity increased with increasing *o*-dichlorobenzene concentration, with the authors reporting significant changes after acute exposures to 200 mg/kg and 300 mg/kg. The authors reported increased necrosis following acute exposure to 300 mg/kg *o*-dichlorobenzene; however, these changes were not statistically significant, possibly due to large variability. In the time-course experiment, the authors observed statistically significant increases in cell proliferation at 2, 3, and 4 days after acute exposure, with the highest increase observed on day 3. The authors observed significant increases in ALT activity on 1, 2, and 3 days after exposure. Day 1 showed the largest increase, with ALT activity decreasing on day 2 and day 3. The authors reported no differences in ALT exposure observed 4 or 7

days post exposure. No statistically significant changes were observed in necrosis at any timepoint following *o*-dichlorobenzene exposure. Based on these findings, the authors concluded that cell proliferation following *o*-dichlorobenzene exposure is compensatory to hepatocellular injury. ALT activity demonstrated a clear dose-response and will be considered for BMD modeling. This study received a high score for overall quality determination.

2.3.2.1.2 10-Day and 90-Day Oral Exposure in Rats (Robinson et al. (1991))

In an oral toxicity study by Robinson et al. (1991), adult male and female SD rats were exposed to *o*-dichlorobenzene via corn oil gavage for either a 10- or 90-day exposure duration. In the 10-day study, rats received doses of 0, 37.5, 75, 150, or 300 mg/kg-day *o*-dichlorobenzene, and in the 90-day study, rats received doses of 0, 25, 100, or 400 mg/kg-day *o*-dichlorobenzene (n = 10/sex/group for both study durations). At the end of each study, authors reported body and organ weights, hematological parameters, serum clinical chemistry levels, and histopathology observations (in the 90-day study, histopathology was only conducted in the control and high-dose treatment groups).

Authors reported multiple organ weight and clinical chemistry effects in males and females of both 10- and 90-day studies relating to liver toxicity effects. In the 10-day oral *o*-dichlorobenzene exposure study, relative liver weight was increased in a dose-dependent manner, with statistically significant increases in males at 300 mg/kg-day (25.8% increase) and females at 150 (21.7% increase) and 300 mg/kg-day (34.5% increase). Comparatively, in the 90-day study, male and female rats also displayed treatment-related dose-dependent increases in relative liver weight, with statistically significant increases in males and females at 100 mg/kg-day (15.2% increase in males and 12.6% increase in females) and 400 mg/kg-day (48% increase in males and 65.4% increase in females). Other relative organ weights were impacted in males (heart, kidney, lung, brain, spleen, testes) and females (kidney) of the 90-day study. Regarding clinical chemistry, serum ALT, a key enzyme indicating liver damage, increased in both sexes in the 10-day 300 mg/kg-day groups (51.1% increase in males and 46.2% increase in females). Increased serum ALT was also increased in the 90-day study in males at 100 and 400 mg/kg-day (103.8% and 61.6% increase, respectively), while a milder, non-significant increase in serum ALT occurred in females. Females of the 10-day study showed statistically significant increased serum cholesterol in all treatment groups; however, this effect did not follow a dose-dependent trend and was not assessed in the 90-day study. Serum BUN, a liver damage biomarker, was not impacted in either sex in the 10-day study but was increased in males at 400 mg/kg-day (25% increase) in the 90-day study. Other serum markers of liver toxicity, including AST, LDH, and albumin, were not impacted.

Authors reported significant treatment-related hepatocellular histopathological effects in both 10- and 90-day studies. Statistically significant increased incidence of hepatocellular necrosis was observed in males but not females of the 10-day study at 300 mg/kg-day (lesion incidence 4/10, slight severity scoring). This effect of single cell necrosis and additional hepatocellular changes of centrilobular degeneration and hypertrophy were statistically significantly increased at 400 mg/kg-day in males and females of the 90-day study (except for necrosis incidence in females). In males, hepatocellular degeneration, hypertrophy, and necrosis occurred at an incidence of 10/10, 9/10, and 7/10, respectively. In females, respective lesion incidence was 8/10, 10/10, and 5/10 (lesion scoring was not reported for either sex).

Liver toxicity effects observed in the oral studies from Robinson et al. (1991) demonstrated dose- and duration-responsive trends, with stronger effects in the high dose group and at 90 days compared to 10 days of exposure. In some cases, increased liver weight and hepatocellular hypertrophy may be considered adaptive and non-adverse responses (Hall et al., 2012). However, accompaniment of these effects with clinical chemistry markers of toxicity (*i.e.*, increased ALT) and further histopathology

indicative of adverse responses (*i.e.*, centrilobular degeneration and hepatocellular necrosis) indicate results by Robinson et al. (1991), particularly at the tested high-doses, cause liver toxicity. Related dose- and duration-response spectrum of effects appeared more cohesive in male rats, with liver weight, serum ALT, and histopathological hepatocellular effects (degeneration, hypertrophy, and single cell necrosis) significantly occurring at levels of 100 mg/kg-day or higher in the 90-day study. Although histopathological results by Robinson et al. (1991) are limited in the 90-day study to the tested high-dose of 400 mg/kg-day, a 90-day oral exposure study by NTP (1985) also reported histopathological effects of hepatocellular degeneration and necrosis at 500 mg/kg-day in male mice. Hepatocyte necrosis lesion incidence was also higher at 90-days compared to 10-days of exposure. Thus, for indicators of liver toxicity at 100 mg/kg-day and above in male rats of the 10- and 90-day studies by Robinson et al. (1991), a NOAEL of 25 mg/kg-day was reported. Given these effects, this study was considered for dose-response analysis of liver toxicity following intermediate exposures.

2.3.2.1.3 13-Week and Chronic Oral Studies of Rats and Mice (NTP, 1985)

In an NTP oral toxicity study (NTP, 1985), adult male and female rats (F344/N) and male and female mice (B6C3F1) were exposed to *o*-dichlorobenzene via corn oil gavage for either a 14-day, 13-week, or two-year exposure duration. In the 14-day study, rats received doses of 0, 60, 125, 250, 500 or 1000 mg/kg-day *o*-dichlorobenzene, and mice received doses of 0, 30, 60, 125, 250 or mg/kg-day *o*-dichlorobenzene. In the 90-day study, rats or mice received doses of 0, 30, 60, 125, or 500 mg/kg-day *o*-dichlorobenzene and (n = 10/sex/group). At the end of each study, authors reported body and organ weights, hematological parameters, serum clinical chemistry levels, and histopathology observations.

Relative liver weight was increased in rats at 125 mg/kg-day and above in both sexes with centrilobular hepatocellular necrosis and degeneration observed at 250 mg/kg-day and above and increased urinary porphyrins at 500 mg/kg-day. Serum cholesterol was increased following as low as 30 mg/kg-day in males, but serum levels of liver enzymes were not increased at any dose. Liver weights were only increased in mice at the highest dose of 500 mg/kg-day (NTP, 1985). Interestingly, while these doses were used as range-finding for a chronic-duration study, no histopathological observations were observed at the highest dose of 120 mg/kg-day administered for 2 years in either species (histopathology not examined at lower doses), indicating that *o*-dichlorobenzene does not induce liver toxicity with longer exposures. Liver weight and clinical chemistry were not measured in the chronic duration experiment.

In an oral study with both 13-week and chronic-duration experiments on mice and rats, relative (but not absolute kidney weight) along with renal tubular degeneration was increased at 13 weeks at the highest dose of 500 mg/kg-day in male rats (NTP, 1985) but not at 250 mg/kg-day. The only clinical chemistry change observed at 13 weeks was increased urine volume in male rats at this same highest dose of 500 mg/kg-day. Non-cancerous tissue changes were not observed in male or female rats exposed up to 120 mg/kg-day in a 2 year bioassay. Renal tubular regeneration increased in a dose-responsive manner in male (but not female) mice given 60 mg/kg-day and 120 mg/kg-day; statistical analysis by ATSDR determined that only the results at 120 mg/kg-day (LOAEL) in male mice were statistically significant (ATSDR, 2006). While renal tubular degeneration was observed in rats at 13 weeks, no significant non-cancer lesions were observed in the kidney at 2 years.

Both oral 90-day studies (Robinson et al., 1991; NTP, 1985) present multiple indications of liver toxicity following approximately the same exposure duration, with a similar LOEL of 100-125 mg/kg-day. Both studies also rated high in data quality evaluation. The NTP study observed additional indicators of toxicity at 250 mg/kg-day and above, while Robinson et al. only examined histopathology at the top dose. The NTP (1985) study had a chronic duration segment, however liver effects were actually most

sensitive in the 13-week segment, perhaps indicating an inverse duration-response (similar to the results for nasal epithelium histopathology in (Zissu, 1995)) and suggesting that the relevance of liver effects from 90-day exposures to chronic duration are uncertain. The liver data from (Robinson et al., 1991) demonstrated a typical duration-response however, with stronger effects at 90 days compared to 10 days. Given these similarities, both studies were used for dose-response analysis of liver toxicity following intermediate exposures.

2.3.2.1.4 Mechanistic and Supporting Evidence

The specific mechanism of liver damage from *o*-dichlorobenzene exposure is unclear. Targeted investigations suggest that liver toxicity from *o*-dichlorobenzene in rats involves reactive oxygen species (Gunawardhana et al., 1993) and is associated with activation of inflammatory signaling cascades through liver macrophage Kupffer cells (Younis et al., 2003; Hoglen et al., 1998). *o*-Dichlorobenzene-induced Kupffer cell activation appears to promote lipid peroxidation and protein adduct formation which correlated with apical indicators of liver injury, with peak response around 24 hours after a single i.p. dose and decreasing thereafter (Hoglen et al., 1998). This time-course may explain the relative sensitivity of acute responses to *o*-dichlorobenzene exposure, as cellular and organ-level oxidative stress and immune responses may equilibrate following sustained long-term exposure.

Experiments on isolated hepatocytes indicate that there are strain-specific differences in this inflammatory signaling response with more muted immune activation in the Fischer-344 (F-344) strain compared to SD (Younis et al., 2003). These data agree with earlier research demonstrating that F-344 rats are significantly more sensitive than SD rats to acute liver toxicity (Stine et al., 1991) (corresponding to a greater rate of tissue repair (Kulkarni et al., 1996)), so differing sensitivities across studies may also be influenced by the specific strain used. These data, however, do not explain the differences seen in the *o*-dichlorobenzene dataset as the more sensitive study. (Robinson et al., 1991) used the seemingly more resistant SD rats while the chronic (NTP, 1985) study used F-344 rats.

A variety of study designs across durations, routes, and lifestages demonstrate that *o*-dichlorobenzene is toxic to rodent livers following acute to intermediate exposures. Interestingly, there is limited evidence of toxicity following chronic exposure, and as with respiratory toxicity there may be an inverse duration-response where acute exposure results in greater toxicity than chronic exposure. Mechanistic evidence for this unique duration-response is provided from data on Kupffer cells indicating a peak in protein adduct formation 24 h after exposure that drops off by 48 h (Hoglen et al., 1998). There are no human studies available, but liver effects seen in multiple species and data supporting various toxicokinetic and molecular signaling mechanisms demonstrate that liver toxicity is unlikely to be species-specific and with multiple contributing modes of action.

In considering the reasonably available information, EPA determines that adverse liver effects are supported by robust animal data demonstrating liver toxicity in both sexes and multiple rodent species and mechanistic evidence for inflammation and oxidative stress along with increased gene expression for multiple cell damage processes. Based on the weight of scientific evidence, evidence integration judgements, and available dose-response data for adverse liver effects, EPA considers the liver toxicity results to be appropriate for conducting dose-response assessment.

2.3.2.2 Oral Study Database Conclusions

Liver effects are the most consistent outcome observed in the oral toxicity database. Adverse liver effects are typically associated with repeated exposure; however, (Umemura et al., 1996) reported dose-responsive increases in serum ALT and necrosis following only a single oral dose. This study compared all three isomers of dichlorobenzene; the *o*- isomer was significantly more acutely toxic than the *p*-

isomer but less toxic than *m*-. Other acute studies reported similar results (Allis et al., 1992; Haskell, 1982) Multiple signs of liver toxicity including increased liver weight, serum biochemistry, and histopathology (only tested at the highest dose) were observed following 10- or 90-days of exposure in (Robinson et al., 1991). These findings are also corroborated by the older (Hollingsworth et al., 1958) and (Rimington and Ziegler, 1963) studies demonstrating liver effects across multiple routes at varying exposure durations. Increased liver weight and histopathology were reported in the 13-week segment of (NTP, 1985), while the two-year segment did not report any adverse liver outcomes. Liver effects from studies with sufficient dose-response information and reporting of definitively adverse effects were further considered for dose-response analysis. The studies selected for dose-response analysis were (Umemura et al., 1996), (Robinson et al., 1991), and (NTP, 1985). EPA additionally considered the tPOD from the 5-day inhalation transcriptomics study (U.S. EPA, 2026l; NIEHS, 2025a) for comparison with the results of the oral studies.

Kidney effects were observed inconsistently in the oral database. Kidney weight increased in (Robinson et al., 1991) and (NTP, 1985). Various histopathology was reported following a very high acute oral dose in (Haskell, 1982) as well as following chronic exposure in (NTP, 1985). While both effects were reported in a two-generation inhalation reproductive toxicity study as well (Biodynamics, 1989), overall these effects were inconsistent across sexes and species. Longer exposures more consistently resulted in some type of kidney outcome; however, the limited observations are insufficient to conclude that an adverse effect would result from human exposure, especially compared to the robust database supporting liver toxicity. Regardless, dose-response analysis of renal tubular degeneration from (NTP, 1985) was performed as a supplemental screening analysis in Appendix L for comparison with results from other assessments (ATSDR, 2006; U.S. EPA, 2006c). Given the uncertainties in the kidney results, EPA considers the kidney toxicity results to be appropriate for conducting screening level evaluations of only long-term, chronic oral or dermal exposure and not for comprehensive risk characterization (see Appendix L). EPA has conducted BMD analysis for some results which can be found in Appendix A.5. The HED of $BMDL_{10}$ = 4.0 mg/kg-day for kidney toxicity is the only POD from a chronic study.

2.3.2.3 Dosimetric and Unit Adjustments for Oral Hazard Values

Dosimetric Adjustments

In the absence of a validated PBPK model or other chemical specific data-derived extrapolation factors (U.S. EPA, 2014), EPA adjusted the HEDs using allometric $BW^{3/4}$ scaling based on guidance from (U.S. EPA, 2011c). 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 2-4.

Equation 2-4 Dosimetric Adjustment Factor (DAF)

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

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)

EPA guidance on allometric scaling (U.S. EPA, 2011c) presents DAFs for extrapolation to humans from several species. However, because those DAFs used a human body weight of 70 kilograms, EPA has

updated the DAFs using a human body weight of 80 kilograms for this risk evaluation (U.S. EPA, 2011a). EPA used the default body weights of 0.25 kg for rats and 0.025 kg for mice from Table B-1 of (U.S. EPA, 2011c). This results in an oral DAF of 0.24 for rats and 0.13 for mice.

Duration Adjustments

Oral values are adjusted to daily exposure by removing the hours/day factor from the equation as shown in Equation 2-5. When a POD is applied to acute exposures, days per week are not adjusted.

Equation 2-5. Adjusting Average Dose or Oral/Dermal POD for Differences in Days Exposure Across Scenarios

$$Dose_{continuous} = Dose_{study} \times (\frac{D_S}{7})$$

Where:

$Dose_{continuous}$	=	Adjusted oral POD
$Dose_{study}$	=	Oral POD from study dataset
D_s	=	Days per week/year exposure in study dataset

2.3.2.3.1 Route-to-Route Extrapolation

EPA additionally considered data from inhalation studies relevant to the key endpoint of liver toxicity. For comparison across routes, route-to-route extrapolation is applied to inhalation values to convert them into internal doses by applying standard exposure factors. Inhalation concentrations for all *o*-dichlorobenzene studies considered for dose-response are reported in parts per million (ppm). It is necessary to interconvert with units of mg/m^3 for purposes of matching exposure estimate outputs or for route-to-route extrapolation. The equations below can be used to interconvert units when needed.

Equation 2-6. Converting from ppm to mg/m^3

$$X\ ppm\ =\ Y\ mg/m^3\ \text{x}\ \frac{24.45^4}{Molecular\ Weight\ (147\frac{g}{mol})}$$

Equation 2-7. Converting from mg/m^3 to ppm

$$X\ mg/m^3\ =\ Y\ ppm\ \text{x}\ \frac{Molecular\ Weight\ (147\frac{g}{mol})}{24.45}$$

Oral values in mg/kg-day and inhalation values in mg/m^3 can then be interconverted based on default exposure factors from (U.S. EPA, 2011a). EPA applies a default body weight of 80 kg and breathing rate of 14.7 m^3/day (based on continuous exposure at rest). OPPT typically assumes 100% oral and inhalation absorption in the absence of data. Dermal absorption is accounted for in the exposure estimate; oral HEDs can therefore be directly applied to the dermal route. HECs and HEDs can be interconverted using Equation 2-8 and the exposure factors above. Respiratory effects are only relevant to the inhalation route and will not be extrapolated to other exposure routes.

[4] The Ideal Gas Law can be used to convert between ppm and mg/m^3. At standard temperature and pressure (STP; 25 °C and 760 mm Hg), 1 mole of gas occupies 24.45 L. However, when conditions differ from STP, a different gas conversion factor can be calculated using the reported experimental temperature or pressure.

Equation 2-8. Converting HEC to HED

$$HED\ (mg/kg) = HEC\ (mg/m^3)\ \text{x}\ \frac{Daily\ Inhalation\ Rate\ (\frac{m^3}{day})}{Body\ Weight\ (kg)}$$

Where:

Daily inhalation rate = 14.7 m^3 for continuous exposure at rest (equal to 0.6125 m^3/h)
Body weight = 80 kg for an average adult

2.3.2.3.2 HED Derivation for Oral PODs

- The HED from (Umemura et al., 1996) is 15.6 mg/kg based on the NOAEL of 120 mg/kg. Because this was a single-dose study and represents acute exposure, duration adjustment was not required. Application of the DAF of 0.13 for mice based on body weight scaling results in the 15.6 mg/kg value (120 mg/kg-day × 0.13 = 15.6 mg/kg-day).
- The calculated HED NOAEL from (Robinson et al., 1991) is 6.0 mg/kg-day based on the NOAEL of 25 mg/kg-day. The study administered *o*-dichlorobenzene daily so a frequency/duration adjustment was not required. The DAF of 0.24 for rats based on body weight scaling was applied to obtain the 6.0 mg/kg-day value (25 mg/kg-day × 0.24 = 6.0 mg/kg-day).
- The liver tPOD of 29.9 ppm from the 6 h/day study was adjusted to an HEC of 7.48 ppm (45 mg/m^3) based on continuous exposure (24 h/day) and a dosimetric adjustment that defaults to 1.0 (29.9 × 6/24 = 7.48 ppm, see Section 2.3.1.4). This value can then be extrapolated to an HED of 8.26 mg/kg-day using the equations in Section 2.3.2.3.1 (7.48 ppm = 45.0 mg/m^3 × 14.7 m^3 / 80 kg = 8.26 mg/kg-day).

2.3.2.4 BMD Modeling of Liver Effects

As previously stated, EPA conducted BMD modeling in accordance with guidance (U.S. EPA, 2012b) to refine PODs for the endpoints considered to be adverse, these studies are described in Section 2.3. See Appendix A for a summary of all BMD modeling results, including model selection and alternative endpoint options. See *Draft Benchmark Dose Modeling Results for o-Dichlorobenzene* (U.S. EPA, 2026b) for full BMD modeling details including statistical tests, results from all models, and any associated graphs. All modeling was performed using EPA's BMDS online modeling suite, and PODs from animal studies were duration- and dosimetrically-adjusted to HECs based on continuous exposure prior to modeling.

The acute oral study (Umemura et al., 1996) examined liver proliferation and toxicity in response to a single gavage exposure for all three dichlorobenzene isomers. The serum liver enzyme ALT was dose-responsively increased with a statistically significant increase at 200 or 300 mg/kg of *o*-dichlorobenzene; necrosis was apparent in approximately 30% of the visualized area following a 300 mg/kg dose. The data was presented in a graph that did not provide precise measurements. Therefore, EPA used software to digitize the graph and determine the values for each bar graph based on the labeled axes (https://plotdigitizer.com/app, accessed January 26, 2026). The digitization results are presented in Appendix A.3.1. The data for ALT was successfully modeled under the assumption of nonconstant variance (modeled failed for constant variance), with the default BMR of 1 SD applied for the continuous dataset. The resulting BMDL was 11.0 mg/kg.

The two repeat-dose oral studies considered for dose-response analysis of liver toxicity were of a similar duration (90 days vs. 13 weeks). EPA modeled both increased liver weight and serum ALT from rats of both sexes in (Robinson et al., 1991), with only male serum ALT failing modeling. Organ weights were all modeled using 10% RD, and female ALT was modeled using a BMR of 1 SD for the continuous

dataset. Increased serum ALT and liver weight were observed at the same dose, indicating that they correspond to adverse liver toxicity and both can be used to identify the toxic threshold.

Similarly, liver weight in rats was modeled from the 13-week results from (NTP, 1985). The results from male rats were not successfully modeled; the results from female rats using the standard 10% RD for liver weight was considered. Other liver effects such as histopathology were only tested at the highest dose and cannot be modeled. Their presence supports the liver toxicity observed in the study, but there is uncertainty in whether the increased liver weight seen at lower doses is adverse without corresponding investigations of histopathology or other indications.

Table 2-7. Dose-Response Analysis of Selected Apical Endpoints from Oral Studies for Deriving Liver Toxicity PODs for Acute/Intermediate Oral/Dermal Exposure Scenarios

Reference and Study Details[a]	Study POD/ Type (HEC)	Effect/Dataset modeled	HED (units)	UF
Umemura et al. (1996) Male B6C3F1 mice exposed to a single dose; 0, 120, 200, 300 mg/kg; acute toxicity study Liver toxicity, oral gavage	NOAEL = 120 (HED = 15.6) mg/kg	Increased serum ALT	$BMDL_{1SD}$ = 11.0 (mg/kg)	UF_A = 3 UF_H = 10 *Total UF = 30*
Robinson et al. (1991) Male and female SD rats; 0, 25, 100, 400 mg/kg; 90-day oral toxicity study Liver toxicity, oral gavage	NOAEL = 25 (HED = 6.0) mg/kg-day	Increased absolute liver weight in males	$BMDL_{10}$ = 27 (mg/kg-day)	UF_A = 3 UF_H = 10 *Total UF = 30*
		Increased relative liver weight in males	$BMDL_{10}$ = 19 (mg/kg-day)	
		Increased absolute liver weight in females	$BMDL_{10}$ = 14 (mg/kg-day)	
		Increased relative liver weight in females	$BMDL_{10}$ = 12 (mg/kg-day)	
		Increased serum ALT in females	$BMDL_{1SD}$ = 19 (mg/kg-day)	
NTP (1985) Male and female F344/N rats and $B6C3F_1$ mice; 0, 30, 60, 125, 250, 500 mg/kg-day; 5 days/week; chronic toxicity/carcinogenicity study Liver toxicity, oral gavage	NOAEL = 60 (HED = 10) mg/kg-day	Increased relative liver weight in female rats	$BMDL_{10}$ = 24 (mg/kg-day)	UF_A = 3 UF_H = 10 *Total UF = 30*

[a] This table only covers apical studies through the oral route. The BMD modeling results and tPOD from NIEHS are considered alongside these results in Section 2.3.2.6.

1. Interspecies Uncertainty Factor (UF_A) of 3

The interspecies UF_A has a toxicokinetics and toxicodynamics component, typically each comprising 3× of the 10× UF_A. As described in Sections 2.3.1.4 and 2.3.2.3, standard dosimetric approaches to account for toxicokinetic differences based on EPA guidance were applied to

derive HEDs. Therefore, only the toxicodynamic component of the UF_A is unaccounted for in the POD value and the remaining UF_A is 3.

2. Intraspecies Uncertainty Factor (UF_H) of 10

EPA uses a default 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*-dichlorobenzene. There is no validated PBPK model available that can quantify human variability in toxicokinetics or toxicodynamics, so the full 10× factor applies to account for both components.

2.3.2.5 Consideration of Exposure Duration(s) Relevant for Risk Characterization

In Section 2.3.1.5, EPA has determined for the inhalation route of exposure that a single hazard value is appropriate. A similar conclusion is drawn for the oral route of exposure. Consistent effects with similar resulting NOAELs and BMDLs were derived from studies ranging from one day to 90 days or 13 weeks (Section 2.3.2.4).

The metabolism profile provides evidence for explaining this unique characteristic of *o*-dichlorobenzene with regards to the duration dependent differences where the acute and short-term studies are more sensitive than the chronic studies. Acute high-level exposure generates reactive metabolites, including epoxide intermediates, at rates that exceed hepatic detoxification capacity, leading to GSH depletion, covalent binding to cellular macromolecules, oxidative stress, and centrilobular hepatocellular injury. In contrast, intermediate or chronic exposure can induce adaptive hepatic responses, such as increased GSH synthesis and upregulation of conjugation pathways, which enhance detoxification and reduce the accumulation of reactive intermediates, thereby attenuating toxicity at comparable dose levels.

Taken together, the apical effects observed across the database of oral studies and the findings following acute exposure does not appear to accumulate greatly across duration, with chronic exposures showing less toxicity than 90 day exposure combined with the metabolic profile support a preliminary conclusion that repeated exposure to *o*-dichlorobenzene via the oral route does not lead to accumulation of effects. As such, EPA is developing a single hazard value relevant for all durations of exposure to *o*-dichlorobenzene (acute, intermediate, and chronic).

2.3.2.6 Hazard Value Derivation Applicable to Oral and Dermal Route of Exposure

As discussed in Section 2.3.1.7, respiratory toxicity is the most appropriate endpoint for risk estimation via the inhalation route of exposure, and those effects are not applicable to other routes. In contrast, systemic effects such as liver toxicity are applicable to all exposure routes. Accordingly, systemic PODs from inhalation studies may also be extrapolated to other routes (Section 2.3.2.3.1). Dermal studies of *o*-dichlorobenzene toxicity are not available, therefore PODs for liver toxicity will be used for the oral and dermal routes.

Several studies identified evidence of liver toxicity, with similar study-derived LOELs ranging from 100 to 200 mg/kg-day (or 100 ppm). These results were relatively consistent between 1 day of exposure and 90 days of exposure. BMD modeling confirmed that the duration-response for liver is relatively flat or even slightly inverted (like respiratory toxicity). The most sensitive apical result based on adverse liver outcomes was a HED $BMDL_{1SD}$ of 11.0 mg/kg for increased serum ALT in mice from the acute (Umemura et al., 1996) study, very similar to the HED $BMDL_{10}$ of 12 mg/kg-day for increase relative liver weight and lower than the HED $BMDL_{1SD}$ for serum ALT in female rats from the 90-day (Robinson et al., 1991) study. Only relative liver weight in female rats could be modeled from (NTP, 1985) with a HED $BMDL_{10}$ of 24 mg/kg-day and other signs of toxicity were either not examined or not

observed at that same dose, however histopathology was seen at the highest dose tested. Interpreting the adversity of outcomes from this study is ambiguous compared to the other two studies, but the HED $BMDL_{10}$ value does further support the consistency of adverse liver outcomes.

In the 5-day inhalation study that was also the source of tPODs (NIEHS, 2025a), serum ALT could not be modeled although the study-derived NOAEC of 100 ppm (converts to 110 mg/kg using the equations in Section 2.3.2.3.1) is very similar to the study-derived NOAEL of 120 mg/kg in (Umemura et al., 1996), suggesting that there is likely not substantial route-specific differential in sensitivity. Comparing BMD modeling results of absolute liver weight across routes (and species) also gives similar results. The HEC $BMCL_{10}$ of 22 ppm (24.3 mg/kg) from mice exposed via inhalation in (NIEHS, 2025a) is comparable to the HED $BMDL_{10}$ of 24 mg/kg-day from rats orally exposed in (NTP, 1985) and the HED $BMDL_{10}$ of 14 or 27 mg/kg-day from rats orally exposed in (NTP, 1985). The liver tPOD (Table 2-5) from (NIEHS, 2025a) was adjusted to an HEC of 7.48 ppm. Respiratory toxicity is the most appropriate POD for inhalation exposures. The apical results indicate that liver toxicity occurs via both inhalation and oral exposure routes. Therefore, the liver tPOD HEC was converted to an HED using the equations in Section 2.3.2.3.1 resulting in an HED of 8.26 mg/kg-day for application to oral and dermal routes.

Table 2-8 presents candidate HED values and associated UFs. All three HEDs have a total UF of 30.

Table 2-8. Candidate Oral/Dermal Liver Toxicity Endpoints

Study	U.S. EPA (2026l); NIEHS (2025a)	Umemura et al. (1996)	Robinson et al. (1991)
Route	*Inhalation*	*Oral*	
Endpoint/ Effects	Mechanism-Informed Superset Gene Expression; Serum ALT	Increased serum ALT, necrosis, and hepatocyte swelling	Increased liver weight, serum ALT, and hepatocyte swelling
Duration	5 days	1 day	90 days
Species	Mice	Mice	Rats
LOAEL	BMDL of 29.87 ppm; serum ALT NOAEL = 110 mg/kg-day	200 mg/kg-day	100 mg/kg-day
NOAEL		120 mg/kg-d	25 mg/kg-day
HED/C-BMDL	tPOD = 8.26 mg/kg-day	11.0 mg/kg-day	12 mg/kg-day
UFs	$UF_A = 3$ $UF_H = 10$ *Total UF = 30*	$UF_A = 3$ $UF_H = 10$ *Total UF = 30*	$UF_A = 3$ $UF_H = 10$ *Total UF = 30*

Given the similarity in the apical PODs and tPOD for liver, the HEDs from (Robinson et al., 1991), (Umemura et al., 1996), and (U.S. EPA, 2026l; NIEHS, 2025a) are considered co-critical with a value of 11.0 mg/kg-day being proposed as the POD for oral/dermal exposure and risk assessment of all exposure durations. There is uncertainty whether the transcriptional responses definitively indicate oncoming adverse outcomes or are merely associated with potential adverse responses. Unlike respiratory toxicity, where there was lower statistical confidence in the apical POD values, Umemara (1996) and Robinson (1991) are both high quality studies that were successfully BMD modeled. Therefore, extrapolating a POD across routes based on transcriptional responses would have lower reliability than those studies. While the POD accounts for the initial cellular processes leading to the downstream adverse outcomes identified in Umemara (1996) and Robinson et al. (1991), the BMDL of 11.0 mg/kg-day from

(Umemura et al., 1996) will be the POD used in the risk evaluation as it falls between the other two values and best represents the liver toxicity of *o*-dichlorobenzene following acute or intermediate exposures.

2.3.2.7 Weight of Scientific Evidence Evaluation for the Oral/Dermal PODs

EPA has preliminarily determined that the HED for *o*-dichlorobenzene of 8.26 mg/kg-day with a total UF of 30 will be used for hazard values for oral and dermal exposures of all durations based on liver effects observed in several studies. EPA has robust confidence in this value based on the following (also see Appendix D):

- Liver effects were reported in multiple high-quality animal studies of varying duration from 1 day through 90 days of exposure.
- Evidence of liver toxicity was reported in both mice and rats, via both oral and inhalation exposure.
- Consistent histopathology (necrosis) and clinical chemistry (liver enzyme ALT) was reported across studies.
- The co-critical PODs from three studies of diverse study design vary by a range of less than 50% from highest to lowest value.
- All three co-critical PODs were successfully BMD modeled, with other supporting BMDLs within a range of less than three-fold.
- There is strong mechanistic support underlying both the apical outcomes and the transcriptomic response.

2.4 Evaluation of the Carcinogenicity of *o*-Dichlorobenzene using the ReCAAP Weight-of-Evidence Framework

2.4.1 Introduction

There is limited epidemiological evidence informing the carcinogenicity of *o*-dichlorobenzene (Section 2.4.2.1). Additionally, an oral cancer bioassay in rats and mice did not achieve the maximum tolerated dose (MTD) and the highest dose was below the non-cancer chronic LOAEL. Accordingly, IARC previously determined that *o*-dichlorobenzene is "*not classifiable as to its carcinogenicity in humans*" and the U.S. EPA previously concluded (in a draft assessment) that there is "*inadequate information to assess carcinogenic potential*." Canada concluded that *o*-dichlorobenzene is "*probably not carcinogenic to man*" based on in adequate evidence suggesting a lack of carcinogenicity in rats and mice in a well-conducted 2 year oral bioassay (Section 2.4.2.3). To evaluate the extent to which the lack of sufficient evidence in the available carcinogenicity studies impart significant uncertainty on the human health risk assessments, EPA has used the ReCAAP framework (Oecd, 2024; Hilton et al., 2022). Although initially developed for agrochemicals, EPA finds that many of the same scientific principles in the ReCAAP Framework apply to TSCA risk evaluations. This framework has also been used in the risk evaluations for DIBP and DCHP (U.S. EPA, 2025a) and was reviewed favorably by the SACC (U.S. EPA, 2025b).

The ReCAAP framework takes into consideration multiple lines of evidence including information pertaining to nomenclature, physical and chemical properties; exposure and use patterns; absorption, distribution, metabolism, and excretion (ADME) properties; and toxicological data (*e.g.*, genetic toxicity, acute toxicity, subchronic toxicity, hormone perturbation, immunotoxicity, MOA). The framework was developed by a workgroup comprised of scientists from academia, government (including EPA), non-governmental organizations, and industry stakeholders. Recently, the Organisation for Economic Co-operation and Development (OECD) has published several Integrated Approach to Testing and Assessment (IATA) case studies demonstrating applicability of the weight of evidence

ReCAAP framework (OECD, 2024). Further demonstrating the applicability of the ReCAAP framework, Goetz et al. (2024) published three retrospective case studies demonstrating application of the ReCAAP Framework for three agrochemical active substances.

Structural analogs were selected for inclusion in the ReCAAP analysis (see Appendix E). In addition to structural similarity, *o*-dichlorobenzene analogs were compared based on physical and chemical properties (Appendix E.3.1) and toxicokinetics (absorption, distribution, metabolism, and excretion) (Appendix E.3.2). Data from *o*-dichlorobenzene and analogs informing direct evidence of carcinogenicity (Section 2.4.2), noncancer health effects in cancer target organs (Section 2.4.3), genotoxicity (Section 2.4.4), and mechanistic data on MOA (Section 2.4.5). Conclusions are provided in Section 2.4.6.

Toxicological data for *o*-dichlorobenzene and potential analogs were gathered from state, federal, and international regulatory assessments (Ntp, 2021; ATSDR, 2020; International Labour, 2018; U.S. EPA, 2018; ATSDR, 2014; U.S. EPA, 2010b, 2009a, b, 2008; ATSDR, 2006; U.S. EPA, 2006b, c, 2003a, b, 2002; IARC, 1999; Health Canada, 1993b, c, d, 1992). Data from these regulatory assessments were not adequate for assessing immune suppression or hormonal perturbations that may contribute to carcinogenicity; therefore, those ReCAAP elements are not addressed below. These considerations are not expected to be relevant to evaluating the available carcinogenicity evidence because the endocrine and immune systems were not identified as critical organ systems for non-cancer hazard outcomes (Section 2.3).

Subchronic and chronic inhalation noncancer toxicity values for the preferred potential analogs (Table_Apx E-3) were obtained from data sources including the U.S. EPA (IRIS, Provisional Peer-Reviewed Toxicity Value [PPRTV] assessments, and Regional Screening Levels [RSLs] for Chemical Contaminants at Superfund Sites), ATSDR, California Environmental Protection Agency (CalEPA) Office of Environmental Health Hazard Assessment (OEHHA), Health Canada, Rijksinstituut voor Volksgezondheid en Milieu (RIVM), and Danish-Derived No-Effect Levels (DNELs). Six analogs with noncancer inhalation toxicity values were identified (*p*-dichlorobenzene, chlorobenzene, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, and 2-chlorotoluene). In addition, searches were conducted for inhalation cancer slope factors and inhalation unit risk values in data sources including U.S. EPA IRIS and PPRTV, CalEPA (OEHHA), Health Canada, and other sources identified in the U.S. EPA CompTox Chemicals Dashboard (Version 2.6.0). One analog with quantitative inhalation cancer values was identified (*p*-dichlorobenzene), however the basis of the cancer value (liver cancer) is not considered relevant to humans (Section 2.4.5).

2.4.2 Evidence of Carcinogenicity

2.4.2.1 Epidemiology Studies

Five epidemiological studies that evaluated cancer outcomes associated with exposure to *o*-dichlorobenzene included general population studies (Von Ehrenstein et al., 2016; Heck et al., 2015; Kato et al., 2004) and limited evaluations of occupational workers (Rodrigues et al., 2020; Chrostek and Thoburn, 1976). Most studies had significant uncertainties in exposure assessment, which limited the interpretation of study findings. *o*-Dichlorobenzene exposure was not quantified (Kato et al., 2004), was below the detection limit (Chrostek and Thoburn, 1976), or was estimated by imprecise means of exposure assessment (*e.g.,* air pollution monitoring data) (Von Ehrenstein et al., 2016; Heck et al., 2015).

Three out of these five cancer studies investigated the adult populations (Rodrigues et al., 2020; Kato et al., 2004; Chrostek and Thoburn, 1976). Due to the lack of *o*-dichlorobenzene exposure data in the exposure assessment for occupational and home pesticides, the risk of *o*-dichlorobenzene exposure causing non-Hodgkin lymphoma among women cannot be calculated (Kato et al., 2004). Malignant neoplasms in the stomach, large intestine, respiratory system, and skin in male workers were not found to be associated with *o*-dichlorobenzene exposure (Chrostek and Thoburn, 1976). A nested case-control study of both male and female workers at semiconductor, electronic module, and storage device manufacturing facilities suggested a possible association between malignant central nervous system tumors and exposure to *o*-dichlorobenzene (estimated using a job-exposure matrix (JEM); 120 cases and 1,028 controls) (Rodrigues et al., 2020). This study was limited by the relatively small number of cases and the potential for exposure misclassification based on the construction of the JEM.

Out of the five cancer studies, two investigated child exposure. Childhood retinoblastoma was not found to be associated with *o*-dichlorobenzene exposure in a case-control study (Heck et al., 2015). Von Ehrenstein (2016) showed that the odds ratio (OR) for the first year of life exposure to *o*-dichlorobenzene and primitive neuroectodermal tumors (PNET) is statistically significant (OR = 3.27; 95% confidence interval (CI): 1.17–9.14), but it only suggested that the infancy exposure may increase the risk of PNET because of the limitation which used air monitoring data in 5-mile radius from residential addresses.

In conclusion, the available human epidemiology studies for *o*-dichlorobenzene are inadequate for assessing carcinogenicity. Human studies for the read-across analogs were also inadequate for assessing carcinogenicity (refer to the discussion of *Weight-of-Evidence Cancer Classifications* below).

2.4.2.2 Animal Studies

A summary of the carcinogenicity data from experimental animal studies for *o*-dichlorobenzene and the read-across analogs is provided in Table 2-9. Generally, no treatment-related neoplastic changes were observed in a chronic oral study of *o*-dichlorobenzene in rats and mice exposed to 60 or 120 mg/kg-day. While previous assessments have indicated that the MTD was not achieved (ATSDR, 2006; U.S. EPA, 2006c), the NTP study (NTP, 1985) showed that there were decreases in survival in both the male and female rats with the males also having decreased body weight compared to the control group throughout most of the study. Both sexes of mice exhibited increased body weight and survival compared to the controls. Thus, the highest dose level in the rat study was close to or overlapped with the MTD and the cancer results in the rat were appropriate to be used in the assessment (Sections 2.3.2, 2.4.3, and 2.3.2.5). A significant trend test was reported for the alveolar/bronchiolar carcinomas in male mice ($p = 0.037$; 4/50, 2/50, and 10/50 in the 0, 60, and 120 mg/kg-day groups, respectively); however, the more appropriate combined incidence of male mice with alveolar/bronchiolar adenomas or carcinomas was not significant in any statistical test (*i.e.,* pair-wise or trend tests) (8/50, 8/50, and 13/50 in the 0, 60, and 120 mg/kg-day groups, respectively) (NTP, 1985). Interestingly in mice, there was a dose-responsive *decrease* in liver adenoma and carcinomas, with a statistically significant decrease in adenomas at the highest dose (using the incidental tumor test and Cochran-Armitage trend test).

A dose-responsive increase of malignant histiocytic lymphoma in mice was reported in that same study. There were also increased adrenal pheochromocytomas only at the middle dose, while the incidence was decreased at the high dose. Therefore, the U.S. EPA concluded that the available oral chronic bioassay for *o*-dichlorobenzene did not demonstrate carcinogenicity at the doses tested (U.S. EPA, 2006c).

No chronic inhalation cancer bioassays were available for *o*-dichlorobenzene. Considering that the respiratory system is the most sensitive target for non-cancer toxicity (Section 2.3), this is an important

data gap for evaluating *o*-dichlorobenzene carcinogenicity. Therefore, analogs with inhalation toxicity data were the focus of the read-across analysis (Appendix E.3).

Table 2-9. Summary of Animal Carcinogenicity Data for *o*-Dichlorobenzene and Read-Across Analogs

Chemical	Oral	Inhalation
***o*-Dichlorobenzene** (CASRN 95-50-1) (ATSDR, 2006; U.S. EPA, 2006c)	*Male and female rats and mice:* • No treatment-related neoplastic changes (MTD was not achieved), except for a statistically significant *reduction* in liver adenomas at the highest dose. • Incidence of bronchoalveolar carcinoma was increased in male mice, but the combined incidence of bronchoalveolar adenoma and carcinoma was not statistically significantly different from controls.	No chronic studies
p-Dichlorobenzene (CASRN 106-46-7) (ATSDR, 2006; U.S. EPA, 2006c)	*Male rats:* • Renal tubular cell adenocarcinoma (Not relevant to humans; mediated by α2μ-globulin, a male rat specific mechanism) • Mononuclear cell leukemia (like historical controls) *Female rats:* No treatment-related neoplastic changes *Male mice:* Hepatocellular adenoma or carcinoma (not relevant to humans; mediated by CAR activation) *Female mice:* Hepatocellular adenoma or carcinoma (not relevant to humans; mediated by CAR activation)	*Male and female rats:* No treatment-related neoplastic changes *Male mice:* Hepatocellular adenoma or carcinoma (not relevant to humans; mediated by CAR activation) *Female mice*: Hepatocellular adenoma or carcinoma (not relevant to humans; mediated by CAR activation) • Lung bronchoalveolar adenoma and carcinomas (female mice only, highest dose only, incidence within historical control range (Aiso et al., 2005))
Chlorobenzene (CASRN 108-90-7) (ATSDR, 2020; U.S. EPA, 2003a)	*Male rats:* Increased incidence of hepatic neoplastic nodules; no liver tumors *Female rats:* No neoplastic nodules or liver tumors *Male and female mice:* No treatment-related neoplastic changes	No inhalation carcinogenicity studies
1,2,4-Trichlorobenzene (CASRN 120-82-1) (ATSDR, 2014; U.S. EPA, 2009b)	*Male and female rats:* No treatment-related neoplastic changes *Male mice:* Hepatocellular adenoma or carcinoma *Female mice:* Hepatocellular adenoma or carcinoma	*Male rats, rabbits, monkeys*: No neoplastic changes were reported in a 26-week study
1,2,3-Trichlorobenzene (CASRN 87-61-6) (ATSDR, 2014; U.S. EPA, 2009a)	No chronic studies	No chronic studies
1,3,5-Trichlorobenzene (CASRN 108-70-3) (ATSDR, 2014)	No chronic studies	No chronic studies
2-Chlorotoluene (CASRN 95-49-8) (U.S. EPA, 2010b)	No chronic studies	No chronic studies

Among the analogs, two chemicals exhibited treatment-related tumors: *p*-dichlorobenzene and 1,2,4-trichlorobenzene. Kidney tumors were observed in male rats in a chronic oral study of *p*-dichlorobenzene (NTP, 1987), but these tumors were not considered relevant to humans because they were mediated by α2μ-globulin (Section 2.4.5) (ATSDR, 2006; U.S. EPA, 2006c). The same study also reported increased mononuclear cell leukemia was also observed in male rats compared to controls; however, the incidence was like historical controls. No neoplastic changes were observed in female rats exposed orally or in male or female rats exposed to *p*-dichlorobenzene via inhalation (ATSDR, 2006; U.S. EPA, 2006c; Aiso et al., 2005).

Liver tumors were observed in male and female mice exposed to *p*-dichlorobenzene (oral and inhalation routes) (ATSDR, 2006; U.S. EPA, 2006c; Aiso et al., 2005; NTP, 1987) and 1,2,4-trichlorobenzene (oral route only) (ATSDR, 2014; U.S. EPA, 2009b). Liver tumors induced by *p*-dichlorobenzene in mice may occur via activation of the constitutive androstane receptor (CAR) (Section 2.4.5); the mechanism by which 1,2,4-trichlorobenzene induces liver tumors is unknown (U.S. EPA, 2009b). A positive trend was observed for the combined incidence of adrenal pheochromocytoma and malignant pheochromocytoma in male mice; however, the incidence was within the historical control range (ATSDR, 2006; U.S. EPA, 2006c). Lung tumors were found at increased incidence in female mice exposed to *p*-dichlorobenzene via inhalation, but the increase was within the historic control range (Aiso et al., 2005). Historical control ranges should ideally be based on a 95% CI instead of the maximum result across all studies, so the significance of falling within the range is muted (Zarn et al., 2024). The incidence of hepatic neoplastic nodules was increased in male rats orally exposed to chlorobenzene; however, no progression to liver tumors was observed (ATSDR, 2020; U.S. EPA, 2003a). No neoplastic nodules or tumors were observed in female rats or male and female mice orally exposed to chlorobenzene. No chronic oral or inhalation cancer bioassays were available for 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, or 2-chlorotoluene (ATSDR, 2014; U.S. EPA, 2010b, 2009a).

2.4.2.3 Cancer Classifications

Cancer weight-of-evidence classifications for *o*-dichlorobenzene and each of the read-across analogs are presented in Table 2-10. Cancer classifications are also provided in Table 2-10 for three additional compounds (1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, and *m*-dichlorobenzene) that were included in the initial- list of 22 preferred potential analogs (Table_Apx E-3). These compounds were not carried forward as candidate analogs due to the lack of inhalation toxicity values (Appendix E.3). Health Canada classified *o*-dichlorobenzene as *probably not carcinogenic to man* based on lack of evidence for carcinogenicity in a two-year oral bioassay in rats and mice (Health Canada, 1996); however, the U.S. EPA determined that there was inadequate information to assess the carcinogenic potential based on lack of human data and questions about the adequacy of the animal data due to uncertainty as to whether or not the MTD had been reached in the two-year bioassay (U.S. EPA, 2006c). Other assessments also determined that *o*-dichlorobenzene was not classifiable as to its carcinogenicity in humans (U.S. EPA, 2018; IARC, 1999).

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Chemical	Agency	Cancer Classification/ Listing	Basis
o-Dichlorobenzene (CASRN 95-50-1)	IARC (1999)	Group 3: not classifiable as to its carcinogenicity in humans	Inadequate evidence in humans; evidence suggesting a lack of carcinogenicity in animals (one oral study in rats and mice)
	U.S. EPA IRIS (U.S. EPA, 2006c)	Inadequate information to assess carcinogenic potential	Lack of human data and questions about the adequacy of the animal data (uncertainty as to whether the MTD had been reached).
	Health Canada (1996, 1993b)	Group V: CEPA (probably not carcinogenic to man)[b]	Inadequate evidence in humans; evidence suggesting lack of carcinogenicity in rats and mice in a well-conducted oral bioassay
	U.S. EPA DWSHA (U.S. EPA, 2018)	Not classifiable as to its carcinogenicity in humans	Basis not reported
p-Dichlorobenzene (CASRN 106-46-7)	U.S. EPA OPPT (U.S. EPA, 2026j)	Not Likely to be carcinogenic to humans by both the oral and inhalation routes	Based on the evidence that liver tumors observed in mice and kidney tumors observed in rats occur via modes of action that are not considered human relevant, and there is insufficient weight of scientific evidence for other cancer types
	NTP RoC (Ntp, 2021)	Reasonably anticipated to be a human carcinogen	Inadequate evidence in humans; sufficient evidence of tumors in mice (liver, adrenal gland) and male rats (kidney, mononuclear-cell leukemia) following oral exposure and mice (liver) following inhalation exposure
	IARC (1999)	Group 2B: possibly carcinogenic to humans	Inadequate evidence in humans; sufficient evidence of liver tumors in male and female mice with supporting mechanistic data indicating potential relevance to humans (DNA damage in liver and spleen of mice and weak DNA binding in mouse liver)
	California OEHHA (Oehha, 2025)	Listed as causing cancer	Basis not reported
	International Labour (2018)	Suspected of causing cancer	Basis not reported
	Health Canada (1996, 1993c)	Group III: CEPA (possibly carcinogenic to humans)	Inadequate evidence in humans; evidence of liver tumors in male and female mice following oral exposure, likely due to non-genotoxic mechanism
	U.S. EPA OPP (U.S. EPA, 2008)	Not likely to be carcinogenic to humans	Based on mitogenesis as a non-mutagenic MOA for liver tumors in mice; carcinogenic effects are not likely at doses not inducing cell proliferation
	U.S. EPA DWSHA (U.S. EPA, 2018)	Possible human carcinogen	Basis not reported
Chlorobenzene (CASRN 108-90-7)	U.S. EPA IRIS (U.S. EPA, 2003a)	D (not classifiable as to human carcinogenicity)	No human data, inadequate animal data, and predominantly negative genetic toxicity data in bacterial, yeast, and mouse lymphoma cells.
	Health Canada (1996, 1992)	Group III: CEPA (possibly carcinogenic to humans)[c]	Inadequate evidence in humans; increased incidence of hepatic neoplastic nodules in male rats following oral exposure

Chemical	Agency	Cancer Classification/ Listing	Basis
	U.S. EPA DWSHA (U.S. EPA, 2018)	Not classifiable as to human carcinogenicity	Basis not reported
1,2,4-Trichlorobenzene (CASRN 120-82-1)	U.S. EPA IRIS (U.S. EPA, 2002)	D (Not classifiable as to human carcinogenicity)	No human data; a dermal study in mice was found inadequate for drawing conclusions as to carcinogenicity in humans
	U.S. EPA PPRTV (U.S. EPA, 2009b)	Likely to be carcinogenic to humans by the oral route of exposure	Based on increased incidence of liver tumors in male and female mice in a two-year dietary study
	Health Canada (1996, 1993d)	Group VI: CEPA (unclassifiable with respect to carcinogenicity to humans)[d]	No human data; a dermal study in mice was inadequate
	U.S. EPA DWSHA (U.S. EPA, 2018)	Not classifiable as to human carcinogenicity	Basis not reported
1,2,3-Trichlorobenzene (CASRN 87-61-6)	U.S. EPA PPRTV (U.S. EPA, 2009a)	Inadequate for assessment of carcinogenic potential	No human or animal studies
	Health Canada (1996, 1993d)	Group VI: CEPA (unclassifiable with respect to carcinogenicity to humans)[d]	No human or animal studies
1,3,5-Trichlorobenzene (CASRN 108-70-3)	Health Canada (1996, 1993d)	Group VI: CEPA (unclassifiable with respect to carcinogenicity to humans)[d]	No human or animal studies
	U.S. EPA DWSHA (U.S. EPA, 2018)	Not classifiable as to human carcinogenicity	Basis not reported
2-Chlorotoluene (CASRN 95-49-8)	U.S. EPA PPRTV (U.S. EPA, 2010b)	Inadequate for assessment of carcinogenic potential	No human or animal studies
	U.S. EPA DWSHA (U.S. EPA, 2018)	Not classifiable as to human carcinogenicity	Basis not reported
1,2,3,4-Tetrachlorobenzene (CASRN 634-66-2)	Health Canada (1996, 1993a)	Group VI: CEPA (unclassifiable with respect to carcinogenicity to humans)[d]	No human data; no adequate animal studies
1,2,3,5-Tetrachlorobenzene (CASRN 634-90-2)	Health Canada (1996, 1993a)	Group VI: CEPA (unclassifiable with respect to carcinogenicity to humans)[d]	No human data; no adequate animal studies
m-Dichlorobenzene (CASRN 541-73-1)	U.S. EPA IRIS (U.S. EPA, 2006c)	Inadequate information to assess carcinogenic potential	Based on no human or animal data
	IARC (1999)	Group 3: Not classifiable as to its	Inadequate evidence in humans and experimental animals

Chemical	Agency	Cancer Classification/ Listing	Basis
		carcinogenicity to humans	
	U.S. EPA DWSHA (U.S. EPA, 2018)	Not classifiable as to human carcinogenicity	Basis not reported

CEPA = Canadian Environmental Protection Act; DNA = deoxyribonucleic acid; DWSHA = Drinking Water Standards and Health Advisories; IARC = International Agency for Research on Cancer; ILO = International Labour Organization; IRIS = Integrated Risk Information System; MTD = maximum tolerated dose; NTP = National Toxicology Program; OEHHA = Office of Environmental Health Hazard Assessment; OPP = Office of Pesticide Programs; PPRTV = Provisional Peer-Revised Toxicity Value; RoC = Report on Carcinogens; U.S. EPA = U.S. Environmental Protection Agency.

[a] Data classifications were available for 9 compounds from the list of the top 22 candidate analogs.
[b] Health Canada (1993b) reported a cancer classification of Group IV (probably not carcinogenic to man); however, the classification scheme was updated for this endpoint under CEPA Health Canada (1996).
[c] Health Canada (1992) reported a cancer classification of Group IIIB: CEPA (possibly carcinogenic to man); however, the classification scheme reported was updated for this endpoint under CEPA (Health Canada, 1996).
[d] Health Canada (1993a, 1993d) reported a cancer classification of Group V (inadequate data for evaluation); however, the classification scheme reported was updated for this endpoint under CEPA (Health Canada, 1996).

Several regulatory assessments classified *p*-dichlorobenzene as a possible/suspected/likely/ anticipated carcinogen (Oehha, 2025; Ntp, 2021; International Labour, 2018; U.S. EPA, 2018, 2006c; IARC, 1999; Health Canada, 1996). However, the current U.S. EPA assessment of *p*-dichlorobenzene concluded that *p*-dichlorobenzene is "Not Likely to be Carcinogenic to Humans", based on the evidence liver tumors observed in mice and kidney tumors observed in rats occur via MOA that are not considered human relevant, and there is insufficient weight of scientific evidence for other cancer types.

The U.S. EPA considered chlorobenzene to be *not classifiable* due to the absence of human data and inadequate animal data (U.S. EPA, 2018, 2003a); however, Health Canada classified chlorobenzene as *possibly carcinogenic to humans* based on the increased incidence of hepatic neoplastic nodules in male rats (Health Canada, 1996, 1992). The U.S. EPA PPRTV Program characterized 1,2,4-trichlorobenzene as *likely to be carcinogenic by the oral route of exposure* based on increased incidence of liver tumors in male and female mice (U.S. EPA, 2009b). Other regulatory assessments considered 1,2,4-trichlorobenzene to be *not classifiable* (U.S. EPA, 2018, 2002; Health Canada, 1996), but these assessments generally preceded the PPRTV document.

Cancer assessments for 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, 2-chlorotoluene, 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, and *m*-dichlorobenzene concluded that the human and experimental animal data were inadequate for assessing carcinogenicity.

2.4.3 Noncancer Health Effects in Cancer Target Organs

As described in Section 2.3, *o*-dichlorobenzene induces noncancer toxicity in the respiratory system, liver, and kidneys, with liver toxicity being the most consistent oral effects and nasal lesions representing the most sensitive inhalation endpoint. Dose-response analysis is described in Section 2.3.1 and 2.3.2 ; this section is to evaluate nonneoplastic changes in a mechanistic context within ReCAAP framework, specifically to assess whether such changes in cancer target organs are likely to serve as precursor events to tumor development. The absence of treatment related tumors in the chronic oral bioassay (60 and 120 mg/kg-day), conducted at doses substantially higher than the noncancer PODs, suggests that tumor induction is unlikely at doses relevant to noncancer risk. Noncancer changes in the target organs in which tumors were observed are discussed below for *o*-dichlorobenzene and analogs that were identified as potentially carcinogenic by one or more agencies (these include *p*-

dichlorobenzene, 1,2,4-trichlorobenzene, and chlorobenzene; see Section 2.4.2). As discussed above, the treatment-related tumors in animals exposed to analogs of *o*-dichlorobenzene (*i.e., p*-dichlorobenzene and 1,2,4-trichlorobenzene) were observed in the livers of mice and in the kidneys of male rats. In addition, while clear evidence of lung tumor induction was not observed for any of the chemicals, a suggestive increase in alveolar/bronchiolar adenomas and/or carcinomas was observed in male mice in the oral study of *o*-dichlorobenzene (NTP, 1985) and in female mice in the inhalation study of *p*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006c). Therefore, nonneoplastic changes in the liver, kidney, and respiratory tract of *o*-dichlorobenzene, *p*-dichlorobenzene, 1,2,4-trichlorobenzene, and chlorobenzene are discussed below.

Additionally, all non-cancer inhalation toxicity values for the selected analogs from government regulatory agencies across the world were also extracted *Draft Candidate Analog and Toxicity Values to Support ReCAAP Analysis for o-Dichlorobenzene* (U.S. EPA, 2026m). The analogs with noncancer inhalation toxicity values are: *p*-dichlorobenzene, chlorobenzene, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, and 2-chlorotoluene. Among the remaining analogs, the only toxicity values linked to specific health effects are for systemic effects (*e.g.,* ataxia, decreased body weight) in 2-chlorotoluene.

2.4.3.1 Liver

A summary of nonneoplastic liver lesions is shown in Table 2-11. *o*-Dichlorobenzene induced effects (*i.e.*, increased liver weight and hepatocellular hypertrophy) consistent with, but not specific to, activation of the CAR (see Section 2.4.5 for further details) and other histopathological changes (*e.g.,* centrilobular degeneration, swelling, and/or necrosis) in both rats and mice following subchronic and/or chronic oral exposure (ATSDR, 2006; U.S. EPA, 2006c). The same spectrum of effects (and a few additional effects, including fatty changes, karyomegaly, vacuolization, and/or atrophy) was observed in rats and mice following exposure to the read-across chemicals *p*-dichlorobenzene and 1,2,4-trichlorobenzene. For *p*-dichlorobenzene and 1,2,4-trichlorobenzene, nonneoplastic effects were observed at chronic oral doses similar to or lower than those associated with liver tumor induction in mice (but not rats) (ATSDR, 2014; U.S. EPA, 2009b; ATSDR, 2006; U.S. EPA, 2006c). Subchronic and chronic oral toxicity data for the read-across chemical chlorobenzene also identify increased liver weight and histopathological effects in rats and mice; the effects identified based on the available (limited) database include hepatocellular degeneration and necrosis, but not hepatocellular hypertrophy (ATSDR, 2020; U.S. EPA, 2006b).

Table 2-11. Nonneoplastic Liver Lesions in Rats and Mice Following Subchronic and Chronic Exposure to *o*-Dichlorobenzene and Read-Across Analogs

Duration	*o*-Dichlorobenzene	*p*-Dichlorobenzene	Chlorobenzene	1,2,4-Trichlorobenzene
Oral				
Subchronic	Rat: • Increased liver weight • Centrilobular hepatocellular hypertrophy and degeneration; single cell necrosis	Rat: • Increased liver weight • Centrilobular hepatocellular hypertrophy; degeneration and necrosis of hepatocytes	Rat: • Increased liver weight • Hepatic degeneration; necrosis	Rat: • Increased liver weight • Hepatocellular hypertrophy; anisokaryosis of hepatocytes; aggregated basophilia and midzonal vacuolization; fatty infiltration
	Mouse: • Increased liver weight	Mouse: • Increased liver weight	Mouse: • Increased liver weight	Mouse: • Increased liver weight

Duration	*o*-Dichlorobenzene	*p*-Dichlorobenzene	Chlorobenzene	1,2,4-Trichlorobenzene
	• Hepatocellular degeneration, centrilobular necrosis, single cell necrosis, pigment deposition	• Centrilobular hepatocellular hypertrophy and degeneration	• Hepatic degeneration; necrosis	• Hepatocellular hypertrophy, atrophy, and vacuolar degeneration; microcytosis, karyomegaly/multinucleation; necrosis
Chronic	Rat: • Increased liver weight • Cloudy swelling	Rat: • Increased liver weight • Cirrhosis; focal necrosis	Rat: • Increased liver weight • Hepatocellular necrosis; unspecified "liver pathology"	Rat: • Increased liver weight • Centrilobular hepatocellular hypertrophy, focal cystic degeneration, diffuse fatty change, periportal cytoplasmic eosinophilic and mild anisokaryosis of hepatocellular nuclei
	Mouse: • No change in liver weight or histology	Mouse: • Hepatocellular hypertrophy, vacuolization, and degeneration; single cell necrosis	Mouse: • No change in liver histology	Mouse: • Increased liver weight • Centrilobular hepatocellular hypertrophy
Inhalation				
Subchronic	Rat: no data	Rat: • Increased liver weight • Centrilobular hepatocellular hypertrophy, congestion, and granular degeneration; cloudy swelling and necrosis	Rat: • Unspecified "slight histological alterations"	Rat: • Increased liver weight • No change in liver histology
	Mouse: no data	Mouse: • Increased liver weight • Centrilobular hepatocellular hypertrophy; necrosis	Mouse: • Fatty degeneration and atrophy	Mouse: no data
Chronic	Rat: • Increased liver weight • Centrilobular hepatocellular hypertrophy	Rat: • Increased liver weight • Centrilobular hepatocellular hypertrophy; cloudy swelling or degeneration of parenchyma; necrosis	Rat: • Increased liver weight • Hepatocellular hypertrophy	Rat: • No change in liver weight or histology
	Mouse: • No change in liver weight or histology	Mouse • Increased liver weight • Centrilobular hepatocellular hypertrophy	Mouse: no data	Mouse: no data
References	ATSDR (2006); U.S. EPA (2006c)	ATSDR (2006); U.S. EPA (2006c)	ATSDR (2020); U.S. EPA (2006b)	ATSDR (2014); U.S. EPA (2009b)

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Only one study was identified that reported liver effects following subchronic or chronic inhalation exposure to *o*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006c). Male and female F0 and F1 rats exposed to greater than or equal to 150 ppm *o*-dichlorobenzene over two generations showed liver effects consistent with CAR activation (increased absolute and relative liver weights and centrilobular hypertrophy). No hepatic effects were noted in rats or mice exposed to lower concentrations (up to 93 or 49 ppm, respectively) for 6–7 months. No subchronic inhalation toxicity studies evaluating hepatic effects in rats or mice were identified. The liver effects observed in rats following exposure to *o*-dichlorobenzene (and additional effects including degeneration, swelling, and/or necrosis) were also observed in both rats and mice following exposure to *p*-dichlorobenzene. The changes in liver weight and histopathology induced by *o*- and *p*-dichlorobenzene were seen at concentrations like those associated with tumor induction in mice (but not rats) chronically exposed to *p*-dichlorobenzene. Increased liver weight and/or hepatocellular hypertrophy were also in rats following subchronic or chronic exposure to chlorobenzene and 1,2,4-trichlorobenzene; one subchronic study in mice exposed to chlorobenzene reported hepatic fatty degeneration and atrophy (ATSDR, 2014; U.S. EPA, 2009b).

While data for *o*-dichlorobenzene and select read-across chemicals show that both rats and mice can develop nonneoplastic hepatic lesions following exposure (including those consistent with CAR activation), these lesions are not necessarily precursors to tumor induction since liver tumors only develop in mice exposed to *p*-dichlorobenzene via the oral and inhalation route (not considered relevant to humans) and 1,2,4-trichlorobenzene via the oral route (Section 2.4.2.2).

2.4.3.2 Kidney

Renal effects were observed following subchronic or chronic oral exposure to *o*-dichlorobenzene; however, findings were inconsistent between species and sexes (ATSDR, 2006; U.S. EPA, 2006c). After 13 weeks, renal tubule degeneration was seen in male rats, but not female rats or mice of either sex. After 103 weeks, renal tubule regeneration was seen in male mice, but not female mice or rats of either sex (Appendix M).

Kidney effects in male rats orally exposed to the isomer *p*-dichlorobenzene are characteristic of α2μ-globulin-mediated toxicity, consistent with the proposed MOA for observed renal tumors. This MOA is considered specific to male rats and is not relevant to humans (U.S. EPA, 2006c) (Section 2.4.5). Observed renal effects in male rats exposed to *p*-dichlorobenzene include hyaline droplet formation, cellular damage, and proliferation of proximal tubule cells in subchronic studies; increased kidney weight, tubular cell hyperplasia, and α2μ-globulin accumulation in a two-generation study; and nephropathy, epithelial hyperplasia of the renal pelvis, mineralization of the collecting tubules in the renal medulla, and focal hyperplasia of renal tubular epithelium in a chronic study (ATSDR, 2006; U.S. EPA, 2006c). Similar effects (*i.e.*, hyaline droplets, granular casts, tubular cell necrosis, cytoplasmic basophilia, papillary mineralization, and urothelial hyperplasia) were observed in male rats in subchronic and chronic inhalation studies of *p*-dichlorobenzene (ATSDR, 2006). Additional kidney effects of subchronic and chronic oral exposure to *p*-dichlorobenzene include increased kidney weights in female rats, tubular degeneration in male rats, and nephropathy and degeneration of cortical tubular epithelium in mice.

Kidney lesions characteristic of α2μ-globulin-mediated toxicity (dilated tubules, granular casts, hyaline droplets, interstitial nephritis) were observed in male rats following subchronic oral exposure to 1,2,4-trichlorobenzene (ATSDR, 2014). Kidney effects were not observed in rats orally exposed to chlorobenzene for up to 2 years (ATSDR, 2020).

Overall, the oral database for *o*-dichlorobenzene provides limited evidence of kidney toxicity in rats and mice. There is inadequate information to determine if nonneoplastic lesions observed in male rats are mediated via an α2μ-globulin MOA consistent with *p*-dichlorobenzene, but this concern is mitigated by the observation of kidney effects in mice as well.

2.4.3.3 Respiratory Tract

A summary of nonneoplastic respiratory lesions is shown in Table 2-12. No treatment-related nonneoplastic lung lesions were observed in any subchronic or chronic oral or inhalation study of *o*-dichlorobenzene in rats or mice. Histopathological evidence of various nasal lesions were observed however in short-term (Zissu, 1995) and subchronic (Cho et al., 2023) studies. Among the read-across analogs, only *p*-dichlorobenzene induced nonneoplastic lung lesions. Interstitial edema, congestion, and alveolar hemorrhage were observed in the lungs of male rats exposed to *p*-dichlorobenzene via inhalation for 16 days (ATSDR, 2006). However, no exposure-related changes in lung histology were reported in rats or mice following subchronic or chronic inhalation exposure to *p*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006c). Similarly, no lung lesions were observed in rats following inhalation exposure to read-across analogs chlorobenzene or 1,2,4-trichlorobenzene; inhalation data are not available for these analogs in mice (ATSDR, 2020; U.S. EPA, 2009b). Oral exposure did not result in pulmonary lesions in rats or mice following exposure to *o*-dichlorobenzene, *p*-dichlorobenzene, 1,2,4-trichlorobenzene, or chlorobenzene (ATSDR, 2020, 2014; U.S. EPA, 2009b; ATSDR, 2006; U.S. EPA, 2006b, c).

Table 2-12. Nonneoplastic Respiratory Lesions in Rats and Mice Following Short-Term, Subchronic, and Chronic Exposure to *o*-Dichlorobenzene and Read-Across Analogs

Duration	*o*-Dichlorobenzene	*p*-Dichlorobenzene	Chlorobenzene	1,2,4-Trichlorobenzene
Inhalation				
Short-term	Rat: No data	Rat: Interstitial edema, congestion, and alveolar hemorrhage in the lungs	Rat: No data	Rat: No data
	Mouse: • Nasal olfactory epithelial lesions • No change in lung histology	Mouse: No data	Mouse: No data	Mouse: No data
Subchronic	Rat: No data	Rat: No change in lung histology	Rat: No data	Rat: No change in lung and/or nasal mucosa histology
	Mouse: • Nasal atrophy, dilation, eosinophilic globules, hyperplasia, metaplasia	Mouse: No change in lung histology	Mouse: No data	Mouse: No data
Chronic	Rat: No change in lung histology	Rat: • Eosinophilic changes in nasal olfactory epithelium and/or respiratory epithelium; respiratory metaplasia of the nasal gland • No change in lung histology	Rat: No change in lung histology	Rat: No change in lung histology
	Mouse:	Mouse:	Mouse: No data	Mouse: No data

Duration	*o*-Dichlorobenzene	*p*-Dichlorobenzene	Chlorobenzene	1,2,4-Trichlorobenzene
	• Histopathological changes in the nasal cavity	• Respiratory metaplasia in the nasal gland and olfactory epithelium • No change in lung histology		
Oral				
Short-term	Rat: No change in lung histology	Rat: No data	Rat: No data	Rat: No change in lung, bronchi, or trachea histology
	Mouse: No data	Mouse: No data	Mouse: No data	Mouse: No data
Subchronic	Rat: No change in lung histology	Rat: • Epithelial necrosis of the nasal turbinates • No change in lung histology	Rat: No change in lung histology	Rat: No change in lung, bronchi, or trachea histology
	Mouse: No change in lung histology	Mouse: No change in lung histology	Mouse: No change in lung histology	Mouse: No change in lung or trachea histology
Chronic	Rat and Mouse: No change in lung histology	Rat and Mouse: No change in lung histology	Rat and Mouse: No change in lung histology	Rat and Mouse: No change in lung or trachea histology
References	ATSDR (2006); U.S. EPA (2006c) and Kim et al. (2025); Cho et al. (2023); Zissu (1995)	ATSDR (2006); U.S. EPA (2006c)	ATSDR (2020); U.S. EPA (2006b)	ATSDR (2014); U.S. EPA (2009b)

o-Dichlorobenzene and *p*-dichlorobenzene induced nasal lesions, but neither *o*-dichlorobenzene nor any of the potentially carcinogenic analogs induced nasal tumors suggesting that observed lesions are not precursors to tumor induction. Moderate-to-severe nasal olfactory epithelial lesions were observed in male mice (only sex tested) exposed to *o*-dichlorobenzene via inhalation for 4 to 14 days (ATSDR, 2006). Nonneoplastic lesions in the nose were also reported in rats and mice following chronic inhalation exposure to *p*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006c). In rats, eosinophilic changes (globules) were observed in nasal olfactory and/or respiratory epithelium and respiratory metaplasia in the nasal gland and/or olfactory epithelium was seen in both rats and mice exposed to *p*-dichlorobenzene via inhalation for 104 weeks (ATSDR, 2006). Nasal lesions (epithelial necrosis of nasal turbinates) were also reported in male and female rats following subchronic oral exposure to *p*-dichlorobenzene (ATSDR, 2006). For other read-across analogs, studies examining the nasal tissues are limited to a subchronic inhalation study for 1,2,4-trichlorobenzene reporting no nasal lesions in rats (ATSDR, 2014).

2.4.4 Genotoxicity and *in vitro* Evidence

Genotoxicity and Mutagenicity

A summary of the U.S. EPA's conclusions regarding the genotoxicity and mutagenicity of *o*-dichlorobenzene and the read-across analogs with non-cancer inhalation toxicity values (Appendix E.3) is shown in Table 2-13.

Table 2-13. Summary of U.S. EPA Conclusions Regarding Genotoxicity and Mutagenicity of *o*-Dichlorobenzene and Read-Across Analogs

Chemical	Genotoxicity Summary
o-Dichlorobenzene (*o*-DCB) (CASRN 95-50-1)	*o*-DCB is not likely to be mutagenic *in vitro* based on predominantly negative results (*e.g.,* in bacterial reverse mutation assays); however, a few positive results were observed for other genotoxicity endpoints. There is limited evidence that *o*-DCB has the potential to induce genotoxic effects (*e.g.,* clastogenicity) *in vivo* (ATSDR, 2006; U.S. EPA, 2006c).
p-Dichlorobenzene (*p*-DCB) (CASRN 106-46-7)	Genotoxicity data for *p*-DCB yielded predominantly negative results *in vitro*; however, positive or equivocal results were observed in mammalian cells. There is some limited evidence that *p*-DCB has the potential to induce genotoxic effects (*e.g.,* clastogenicity) *in vivo* (ATSDR, 2006; U.S. EPA, 2006c). Genotoxicity data for *p*-DCB yielded predominantly negative results *in vitro*; however, positive or equivocal results were observed in mammalian cells. There is some limited evidence that *p*-DCB has the potential to induce genotoxic effects (*e.g.,* clastogenicity) *in vivo* (ATSDR, 2006; U.S. EPA, 2006c).
Chlorobenzene (CASRN 108-90-7)	Predominantly negative for mutagenicity *in vitro*; has the potential to induce genotoxic effects (*e.g.,* clastogenicity) *in vivo* (ATSDR, 2020; U.S. EPA, 2003a). Predominantly negative for mutagenicity *in vitro*; has the potential to induce genotoxic effects (*e.g.,* clastogenicity) *in vivo* (ATSDR, 2020; U.S. EPA, 2003a).
1,2,4-Trichlorobenzene (CASRN 120-82-1)	Not likely to be mutagenic or clastogenic *in vitro*; evidence indicates that trichlorobenzenes are clastogenic *in vivo* (ATSDR, 2014; U.S. EPA, 2009b). Not likely to be mutagenic or clastogenic *in vitro*; evidence indicates that trichlorobenzenes are clastogenic *in vivo* (ATSDR, 2014; U.S. EPA, 2009b).
1,2,3-Trichlorobenzene (CASRN 87-61-6)	Not likely to be mutagenic or clastogenic *in vitro*; evidence indicates that trichlorobenzenes are clastogenic *in vivo* (ATSDR, 2014; U.S. EPA, 2009a). Not likely to be mutagenic or clastogenic *in vitro*; evidence indicates that trichlorobenzenes are clastogenic *in vivo* (ATSDR, 2014; U.S. EPA, 2009a).
1,3,5-Trichlorobenzene (CASRN 108-70-3)	Not likely to be mutagenic or clastogenic *in vitro*; evidence indicates that trichlorobenzenes are clastogenic *in vivo* (ATSDR, 2014). Not likely to be mutagenic or clastogenic *in vitro*; evidence indicates that trichlorobenzenes are clastogenic *in vivo* (ATSDR, 2014).
2-Chlorotoluene (CASRN 95-49-8)	Very limited genotoxicity data *in vitro* suggest that 2-chlorotoluene is not mutagenic *in vitro* (no data on other genotoxicity endpoints were identified *in vitro* or *in vivo*) (U.S. EPA, 2010b).

Various genotoxicity endpoints have been evaluated for *o*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006c). In several studies, *o*-dichlorobenzene showed no mutagenic activity in bacterial assays with or without activation, but induced mutations, in the presence of activation only, in *Saccharomyces cerevisiae*. *o*-Dichlorobenzene also yielded positive results in assays of DNA damage in bacteria and yeast (activation was not required) but was generally negative for other genotoxicity endpoints (*e.g.,* induction of *umu* genes in *Salmonella typhimurium* and prophage lambda in *Escherichia coli*). In mammalian cells, *o*-dichlorobenzene induced forward mutation in mouse lymphoma cells in the presence of activation. With respect to clastogenicity, *o*-dichlorobenzene was positive for sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells in the presence of activation, but there was no evidence of chromosomal aberrations (CAs) in the same assay. *o*-Dichlorobenzene was also negative for DNA repair in rat hepatocytes and replicative DNA synthesis in human lymphocytes regardless of activation status. Limited *in vivo* data showed a dose-related increase in micronuclei (MN) formation in the bone marrow of mice treated with *o*-dichlorobenzene via i.p. injection. *o*-Dichlorobenzene was shown to weakly bind macromolecules (DNA, ribonucleic acid [RNA], and protein) in the tissues of treated rats and mice (*e.g.,* liver, lung, and kidney). An analysis of 26 workers accidentally exposed to *o*-dichlorobenzene for 4 days reported significantly increased CAs in peripheral

blood lymphocytes relative to controls; no information on prior or subsequent exposure was reported (IARC, 1999).

The scope of genotoxicity studies evaluated for *p*-dichlorobenzene overlaps with *o*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006c). In several studies, *p*-dichlorobenzene did not induce reverse mutations in bacteria or DNA damage in bacteria or yeast; mutations in *S. cerevisiae* were observed in a single study. Results from mutagenicity assays in mammalian cells showed positive or equivocal results for forward mutation in mouse lymphoma cells (both in the presence and absence of metabolic activation). Results were mixed for clastogenicity in mammalian cells *in vitro*: *p*-dichlorobenzene failed to induce CAs or SCEs in CHO cells, was positive for MN (and DNA damage) in rat and human kidney cells with activation and was equivocal for MN in human and rat hepatocytes. Unscheduled DNA synthesis (UDS) was not observed in human cells (lymphocytes or HeLa cells) treated with *p*-dichlorobenzene. *In vivo*, rats exposed to *p*-dichlorobenzene via inhalation did not show CAs in bone marrow cells; however, conflicting (positive and negative) results were observed for MN in mouse bone marrow erythrocytes. *p*-Dichlorobenzene did not elicit UDS in mouse hepatocytes or rat kidney cells; however, *p*-dichlorobenzene was positive for MN, DNA damage, and replicative DNA synthesis in the rat (kidney) and replicative DNA synthesis in the mouse (liver).

Data on the genotoxicity of chlorobenzene are more limited (ATSDR, 2020; U.S. EPA, 2003a). Chlorobenzene was negative for reverse mutation and DNA damage in microbial cells in several studies; results for mutagenicity in mouse lymphoma cells were mixed in two studies. In mammalian cells *in vitro*, positive results for clastogenicity were seen for SCEs in CHO cells; chlorobenzene was negative for CAs in CHO cells in two studies. Chlorobenzene was also negative for DNA repair and cell transformation in rat liver cells. *In vivo*, multiple i.p. studies evaluated clastogenicity in rat and mouse bone marrow; these studies showed positive results for CAs, and mixed results for MN in both species. Likewise, conflicting results were seen in two studies that assayed DNA damage in mice following i.p. injection (positive in peripheral lymphocytes but negative in the bone marrow). There was no evidence of sex-linked recessive mutations in the male germ cells of *Drosophila* exposed to chlorobenzene.

Genotoxicity data for the trichlorobenzenes vary by isomer; however, data for all three trichlorobenzene read-across chemicals (1,2,4-, 1,2,3-, and 1,3,5-trichlorobenzene) show predominantly negative results in bacterial reverse mutation assays in the presence and absence of metabolic activation; only the 1,3,5-isomer was weakly positive for mitotic recombination in *S. cerevisiae* (ATSDR, 2014; U.S. EPA, 2009b 1257695). In mammalian cells, 1,2,3- and 1,2,4-trichlorobenzene were positive and 1,3,5-trichlorobenzene was weakly positive for cell replication and DNA damage in Chinese hamster V79 cells in the absence of activation. 1,2,4-Trichlorobenzene tested negative in a DNA repair assay in rat hepatocytes but was positive in a cell transformation assay in rat liver epithelial cells (in the absence of activation but at concentrations that elicited cytotoxicity). With respect to clastogenicity, *in vitro* tests showed that the 1,2,3- and 1,2,4-isomers did not induce CAs in CHO or Chinese hamster lung (CHL) cells in the presence or absence of activation. However, two *in vivo* tests that exposed different strains of mice via i.p. injection showed that the three trichlorobenzenes tested positive for MN in the bone marrow. There was no evidence of sex-linked recessive mutations in the male germ cells of *Drosophila* exposed to 1,3,5-trichlorobenzene.

2-Chlorotoluene genotoxicity data are limited to a few *in vitro* studies, which showed that this chemical was negative for reverse mutations in *S. typhimurium* and negative for forward mutations in mouse lymphoma cells in the presence and absence of metabolic activation (U.S. EPA, 2010b).

Other in vitro Cancer Evidence

The cell transformation assay is an *in vitro* system using immortalized cell lines growing to confluence (*i.e.,* full monolayer density). One hallmark of transformed cell populations can be observed in their ability to evade traditional growth inhibition signals. In this assay, growth past confluence can be measured by quantifying the number and size of transformed cell colonies that grow on top of the monolayer. There are several versions of this assay including using different cell lines and for measuring either initiation or promotion. They can therefore be used to screen for promotion of transformed cell characteristics of genotoxic or non-genotoxic carcinogens, with strong concordance established between cell transformation and rodent cancer bioassay results (Sakai et al., 2011). A recent study using Bhas 42 cells did not observe an increase in the ability of cells to appreciably grow post confluence following incubation with *o*-dichlorobenzene (Lim and Seo, 2024). There was also an absence of DNA methylation indicating that *o*-dichlorobenzene does not exhibit epigenetic gene silencing.

2.4.5 Mechanistic Studies and Considerations to Support a Proposed Mode of Action (MOA) for Carcinogenicity

Liver Tumors in Mice

A proposed MOA for liver tumors in mice exposed to *p*-dichlorobenzene involves activation of the CAR as the MIE followed by altered gene expression in hepatocytes, hepatocellular hypertrophy, sustained hepatocyte proliferation, and inhibition of apoptosis. These cellular responses may lead to the formation of preneoplastic liver foci and hepatocellular adenoma and carcinoma. This MOA is supported by evidence for CAR activation in a transcriptional profiling study in wild-type and genetically modified mice orally exposed to 600 mg/kg-day for 3 days (U.S. EPA, 2026h). The key findings from this study are as follows:

- In wild-type mice, exposure to *p*-dichlorobenzene induces *Cyp2b10*, marker consistent with CAR activation, and increases the gene expression of CAR targets (*e.g., Ugt1a1, Gstm3, Akr1b7*), providing mechanistic evidence supporting CAR activation. There was negligible induction of *Cyp3a11*, which is a marker of the pregnane X receptor (PXR) activation.
- Humanized mice showed a similar, but smaller response to *p*-dichlorobenzene exposure as compared to wild-type mice.
- No induction of CAR/PXR targets was observed in CAR/PXR double-knockout mice exposed to *p*-dichlorobenzene.

BrdU labeling studies in rodents also suggest a role for sustained cell proliferation in *p*-dichlorobenzene-induced liver cancer (U.S. EPA, 2006c). These data suggest that CAR activation may play a role in the MOA for *p*-dichlorobenzene-induced liver tumors in mice.

Transcriptomic Analysis in Rats: EPA additionally performed transcriptomic analysis on rat iver using gene expression biomarkers that predict activation of MIEs that lead to rat liver tumors. As presented in Appendix F, one existing rat study ((Consortium, 2010; Popovici et al., 2010), GSE24363) was identified in the literature and existing databases. In this study, male rats were exposed to 15, 150, and 1,500 mg/kg-day for up to 2 days. Seven biomarkers were used to determine if MIEs were altered by treatment. The biomarker for cytotoxicity was elevated at 6 hours after exposure to 15 mg/kg and at 1 day at 150 mg/kg. Markers for other non-genotoxic cancer mechanisms (AHR, CAR) were elevated at 150. While AHR, CAR, and estrogen receptor were elevated at 1,500 mg/kg, the results were not considered due to the high levels of exposure. The cytotoxicity biomarker results are consistent with the observed pattern of acute liver toxicity and reduced sensitivity following continued exposure, leading ultimately to a negative association between chronic *o*-dichlorobenzene exposure and liver cancer.

The NIEHS study in which rats were exposed to inhaled *o*-dichlorobenzene for 5 days was used to determine if endocrine-related signaling was perturbed by examining gene expression changes in the liver. (U.S. EPA, 2026l) demonstrated that there was minimal evidence of estrogen receptor transcriptional activity and only at the highest concentration of exposure. No significantly disrupted pathways were observed that included terms for any endocrine-related pathways. However, it is difficult to ascertain whether this is due to the limited transcriptomic coverage on the S1500+ platform or an absence of effect. Analysis of the same data focusing on CAR activation and CAR-related pathways revealed that *o*-dichlorobenzene caused some concentration-responsive activation of CAR biomarker genes in both mouse and rat liver, demonstrating that hepatic CAR activation occurs following inhaled 5-day exposure to *o*-dichlorobenzene and can be found in Appendix D of the *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l).

Inadequate data are available for assessing the MOA for 1,2,4-trichlorobenzene-induced liver tumors (U.S. EPA, 2009b).

Kidney Tumors in Male Rats

The kidney tumors in male rats exposed to *p*-dichlorobenzene by gavage for 103 weeks are consistent with an α2μ-globulin-mediated MOA (U.S. EPA, 2006c). The key findings related to this MOA are as follows:

- In chronic studies, renal tumors were observed in male rats, but not in female rats or mice of either sex.
- Renal lesions (*i.e.*, hyaline droplet formation, cellular damage, proliferation of proximal tubule cells) in subchronic studies occurred in male rats only and were characteristic of α2μ-globulin toxicity.
- In a two-generation study, male rat-specific renal effects included increased kidney weight, tubular cell hyperplasia, and α2μ-globulin accumulation.
- No renal effects were observed in NBR rats, a strain that does not synthesize α2μ-globulin. Similar exposure to Fischer 344 rats showed α2μ-globulin accumulation and renal toxicity.
- Increased cell proliferation was measured by BrdU labeling in proximal tubule cells of male rats exposed to *p*-dichlorobenzene for 4 or 13 weeks.
- The protein accumulating in tubular cells in male rats was identified as α2μ-globulin.
- *p*-Dichlorobenzene and its metabolite, 2,5-DCP, bind reversibly to α2μ-globulin.

These findings establish an α2μ-globulin-mediated MOA for kidney tumors, which is considered to be specific to male rats and not relevant to humans (Swenberg, 1993; U.S. EPA, 1991a). Due to dose limitations in the chronic oral bioassay for *o*-dichlorobenzene (NTP, 1985), it is not possible to determine whether kidney tumors could be induced in male rats via an α2μ-globulin-mediated MOA.

Other Cancers

Inadequate mechanistic evidence is available for other cancer types. Potential MOAs based on limited evidence include inflammation, oxidative stress, and regenerative proliferation. Gene sets associated with apoptosis, cytotoxicity, inflammation, oxidative stress, and/or necrosis in lung were elevated only at the highest concentration (Section 2.3.1.1.3) and to a much greater extent in mice. Gene signatures for endocrine-relevant signaling did not demonstrate any significant induction following *o*-dichlorobenzene exposure in lungs of mice or rats (U.S. EPA, 2026l).

2.4.6 Weight-of-Scientific-Evidence Conclusions Regarding Carcinogenicity of *o*-Dichlorobenzene Based on Read-across

The goal of this ReCAAP analysis was to use data for *o*-dichlorobenzene and its read-across analogs to evaluate the extent to which the lack of high dose oral or inhalation carcinogenicity studies imparts significant uncertainty on the human health risk assessments. Candidate structural analogs were identified using multiple resources for analog searching and refinement using chemistry expertise (Appendix E). Assessment of the suitability of the analogs revealed that physical-chemical and toxicokinetic properties were similar for *o*-dichlorobenzene and the candidate read-across analogs:

- All compounds had similar molecular weight and log K_{ow} values and each exhibited moderate-to-high vapor pressure and moderate water solubility.
- Following oral and inhalation exposure, *o*-dichlorobenzene and the read-across analog compounds are rapidly absorbed, distributed, metabolized, and excreted primarily in the urine with minor amounts excreted in feces and expired air. Although these compounds partition to fat, the rapid excretion profiles suggest that they will not bioaccumulate.
- Metabolic pathways are similar for *o*-dichlorobenzene and most read-across analogs except for 2-chlorotoluene, which undergoes methyl group oxidation.

These comparisons indicate that the selected analogs are suitable for read-across to *o*-dichlorobenzene. Among the six analogs, only three had data on potential carcinogenicity (*p*-dichlorobenzene, 1,2,4-trichlorobenzene, and chlorobenzene); thus, comparisons of toxicity and MOA information are primarily between *o*-dichlorobenzene and these three compounds. Based on the following findings from data on *o*-dichlorobenzene and the read-across analogs, the U.S. EPA has determined that there is sufficient evidence for evaluating the carcinogenicity of *o*-dichlorobenzene based on the reasonably available information, summarized below:

- A chronic oral cancer bioassay of *o*-dichlorobenzene in rats and mice exposed by gavage showed no treatment-related increase in the incidence of any neoplasm; however, the MTD was not achieved. Liver tumors were *decreased* in exposed groups compared to controls.
- *o*-Dichlorobenzene and the read-across analogs are generally negative for mutagenicity; these compounds do exhibit clastogenicity *in vivo*. *o*-Dichlorobenzene was also negative in an *in vitro* cell transformation assay.
- The available human epidemiology studies for *o*-dichlorobenzene and the read-across analogs are inadequate for assessing carcinogenicity.
- Liver tumors in male and female mice induced by *p*-dichlorobenzene (analog of *o*-dichlorobenzene) may not be relevant to humans because they are likely to be mediated by CAR activation (U.S. EPA, 2026h). Data are not available to evaluate the MOA for liver tumors in mice induced by 1,2,4-trichlorobenzene (analog of *o*-dichlorobenzene). *o*-Dichlorobenzene induced nonneoplastic liver lesions consistent with (but not specific to) CAR activation, along with other histopathological hepatic changes. However, since tumors were observed only in mice exposed to *p*-dichlorobenzene and 1,2,4-trichlorobenzene and nonneoplastic liver lesions were seen in both rats and mice, observed lesions in *o*-dichlorobenzene are not necessarily precursors to tumor induction.
- Transcriptomic analysis using gene expression biomarkers supports the observed pattern of acute non-cancer toxicity in the absence of chronic adverse liver outcomes, including cancer.
- Kidney tumors induced by *p*-dichlorobenzene (analog of *o*-dichlorobenzene) are not relevant to humans because they are mediated by α2μ-globulin. The oral database for *o*-dichlorobenzene provides limited evidence of kidney toxicity in rats and mice. Due to dose limitations in the chronic oral bioassay for *o*-dichlorobenzene, it is not possible to determine whether kidney tumors could be induced in male rats via an α2μ-globulin-mediated MOA.

- Lung tumors (bronchoalveolar adenoma and carcinomas) were found in male mice exposed orally to *o*-dichlorobenzene and female mice exposed to *p*-dichlorobenzene via inhalation; however, the increases were marginally significant and/or within the historic control range. This increase was not observed in rats; in mice the lung tumors were not increased for females exposed orally to *o*-dichlorobenzene or for males exposed via inhalation to *p*-dichlorobenzene.
- Nonneoplastic nasal lesions were observed in mice and rats exposed to *p*-dichlorobenzene in chronic inhalation studies and across multiple exposure durations for *o*-dichlorobenzene; however, no evidence of nasal tumors was reported in chronic studies for either isomer, so these lesions do not appear to be preneoplastic.
- Cancer assessments for *o*-dichlorobenzene, 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, and 2-chlorotoluene concluded that the human and experimental animal data were inadequate for assessing carcinogenicity. *p*-Dichlorobenzene was previously classified as a possible or suspected carcinogen; however, recent consideration of the suspected CAR-mediated MOA suggests that this compound is not likely to be carcinogenic in humans (U.S. EPA, 2026h). 1,2,4-Trichlorobenzene was classified as likely to be carcinogenic by the oral route based on liver tumors in mice; the MOA for this chemical is unknown. While the U.S. EPA characterized the human and animal data for chlorobenzene as inadequate to assess carcinogenicity, Health Canada classified it as *possibly carcinogenic to humans* based on hepatic neoplastic nodules in rats, even though no progression to tumors was observed.

Based on the weight of scientific evidence, the U.S. EPA has concluded that quantitative cancer risk assessment of *o*-dichlorobenzene is not warranted and any additional cancer bioassays would be unlikely to provide evidence leading to a different conclusion. The non-cancer PODs identified in this document are considered protective of human health, including protective for potential carcinogenic effects. While there is uncertainty regarding the relevance to *o*-dichlorobenzene of liver tumors induced by oral exposure to 1,2,4-trichlorobenzene, the absence of liver tumors in the chronic oral study of *o*-dichlorobenzene, despite its failure to reach MTD, provides some assurance that such cancers are not induced in the dose range tested in that study.

2.5 Consideration of PESS and Aggregate Exposure

2.5.1 Hazard Considerations for Aggregate Exposure

EPA has defined aggregate exposure as "the combined exposures from a chemical substance across multiple routes and across multiple pathways" (89 FR 37028, May 3, 2024, to be codified at 40 CFR 702.33). Aggregation across routes could be biologically relevant for oral and dermal routes, which will use the same PODs. However, given the physical-chemical properties of *o*-dichlorobenzene (U.S. EPA, 2026c) and the limited dermal absorption (Section 2.2.1), non-inhalation routes are likely to be very minor contributors to the overall risk for the chemical. Therefore, aggregation across routes is not expected to be relevant except when inhalation exposure is not present.

2.5.2 PESS Based on Greater Susceptibility

In this section, EPA addresses subpopulations expected to be more susceptible to *o*-dichlorobenzene exposure than other populations. Table 2-14 presents the data sources that were used in the PESS analysis that evaluated susceptible subpopulations and identifies whether and how the subpopulation was addressed quantitatively in the risk evaluation of *o*-dichlorobenzene.

EPA examined sources of biological susceptibility for each of the susceptibility factors in Table 2-14. The Agency quantitatively incorporated these considerations into hazard values and subsequent risk estimates when possible; however, for many factors, EPA did not identify any reasonably available

information to support quantitative adjustment of hazard/risk values. For these other factors, the Agency acknowledges either direct or indirect information suggesting additional susceptibility of certain subpopulations (https://www.epa.gov/expobox/exposure-assessment-tools-lifestages-and-populations-highly-exposed-or-other-susceptible, accessed December 29, 2025).

EPA used the most sensitive effects from rodent assays (including reproductive/developmental toxicity tests) for non-cancer dose-response modeling. A 10× UF_H was applied to account for human toxicokinetic and toxicodynamic variability, which is expected to account for considerations such as genetic polymorphisms and existing disease states.

Table 2-14. PESS Evidence Crosswalk for Biological Susceptibility Considerations

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to *o*-Dichlorobenzene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to *o*-Dichlorobenzene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	Decreased pup weights were observed in a two-generation inhalation study that included exposure during gestation and lactation.	Biodynamics (1989)			The most sensitive effects from rodent assays were used for non-cancer dose-response modeling; effects in adult animals identified lower PODs than those identified for developmental effects.
	Pregnancy/ lactating status	Decreased maternal body weight and/or body weight gain were observed in gestational and two-generation inhalation studies; however, pregnant dams did not appear more susceptible than males.	Biodynamics (1989); Hayes et al. (1985)			The most sensitive effects from rodent assays were used for non-cancer dose-response modeling; there was no evidence of increased susceptibility in pregnant animals.
	Males of reproductive age	Decreased body weights were consistently observed in males in a two-generation inhalation study.	Biodynamics (1989)			The most sensitive effects from rodent assays were used for non-cancer dose-response modeling.
	Children	No direct evidence identified.		Childhood is considered a sensitive lifestage due to the continued development of various organ systems	U.S. EPA (1991b)	This susceptibility is expected to be covered by the 10× UF_H.
	Elderly	No direct evidence identified.		Lung function is reduced in the elderly, making them more susceptible to respiratory effects induced by *o*-dichlorobenzene.	Luoto et al. (2019)	This susceptibility is expected to be covered by the 10× UF_H.
Pre-existing disease or disorder	Health outcome/ target organs	No direct evidence identified.		Any pre-existing condition affecting a target organ will increase susceptibility to toxicity induced by *o*-dichlorobenzene in that organ.		This susceptibility is expected to be covered by the 10× UF_H.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to *o*-Dichlorobenzene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to *o*-Dichlorobenzene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Toxicokinetics	One study suggests that inducers of cytochrome P450 (CYP450) enzymes may exacerbate liver and/or kidney toxicity induced by *o*-dichlorobenzene.	Valentovic et al. (1993)	Higher rates of metabolism resulting in increased reactive metabolites or GSH depletion would increase susceptibility, especially for liver and kidney toxicity (*i.e.*, sites of metabolism and excretion).	ATSDR (2006)	This susceptibility is expected to be covered by the 10× UF_H.
Lifestyle activities	Smoking	No direct evidence identified.		A regular smoking habit or exposure to second-hand tobacco smoke has a clear effect on respiratory function, especially in younger lifestages.	Chatzimicael et al. (2008); Vianna et al. (2008)	No direct evidence available.
	Alcohol consumption	No direct evidence identified.		Alcohol consumption and alcoholism are well-established to be associated with liver disease.	Crabb (1993)	No direct evidence available.
	Physical activity	No direct evidence identified.		Insufficient activity may increase susceptibility to multiple health outcomes. Overly strenuous activity may also increase susceptibility.	CDC (2022)	No direct evidence available.
Sociodemo-graphics	Sex	Health effects observed following oral or inhalation exposure often demonstrated differential sensitivity across sexes.	Cho et al. (2023); Robinson et al. (1991); Biodynamics (1989); NTP (1985), Section 2.3			Either the most sensitive sex from rodent assays was used for non-cancer dose-response modeling, or if both sexes were modeled, the most sensitive POD was selected for use in risk estimation.
Nutrition	Diet	*o*-Dichlorobenzene distributes to adipose tissue, suggesting that obesity may result in higher body burden for a given exposure.	Section 2.2.2			Quantitative data for this chemical is not available based on differing eating habits or obesity.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to *o*-Dichlorobenzene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to *o*-Dichlorobenzene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Malnutrition	No direct evidence identified.		Micronutrient malnutrition can lead to multiple developmental outcomes that include birth defects, maternal and infant deaths, low birth weight, and poor fetal growth, among others.	CDC (2023)	No direct evidence available.
Genetics/ epigenetics	Health outcome/ target organs	Different rat strains demonstrate differential sensitivity to liver toxicity associated with variations in immune response.	Younis et al. (2003); Kulkarni et al. (1996); Stine et al. (1991)	Genetic and environmental factors affect gene expression promoting respiratory immune responses. Any polymorphism or mutation impacting biological functions for critical organ systems would impact susceptibility to *o*-dichlorobenzene.	Tarlo (2006)	The most sensitive and robust animal toxicity outcomes across species and strains were used for deriving hazard values.
	Toxicokinetics	No direct evidence identified.		Higher rates of metabolism resulting in increased reactive metabolites or GSH depletion would increase susceptibility, especially for liver and kidney toxicity (*i.e.*, sites of metabolism and excretion).	ATSDR (2006)	This susceptibility is expected to be covered by the 10× UF_H.
Other chemical and nonchemical stressors	Built environment	No direct evidence identified.		Poor-quality housing is associated with a variety of negative health outcomes.	ODPHP (2023a)	No direct evidence available.
	Social environment	No direct evidence identified.		Social isolation and other social determinants (*e.g.,* decreased social capital, stress) can lead to negative health outcomes.	ODPHP (2023b)	No direct evidence available.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to *o*-Dichlorobenzene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to *o*-Dichlorobenzene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Chemical co-exposures	An epidemiology study identified an association between exposure to organochlorine compounds (including *o*-dichlorobenzene) and higher immunoglobulin E (IgE) levels (as a marker of allergic sensitization).	Reichrtová et al. (1999)	Exposure to other chemicals that impact the same organ systems could result in additive or synergistic toxicity.	U.S. EPA (2003c, 1986)	No direct evidence available.

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2.6 Human Health Hazard Assessment Conclusions

In this human health assessment of *o*-dichlorobenzene, EPA derived distinct non-cancer PODs for inhalation and oral/dermal routes applied to each duration category to be used for risk estimation. Confidence in these PODs is strengthened by the analysis of endpoint-specific transcriptional responses corresponding to each organ system, which resulted in derivation of tPODs like but lower than the respective apical PODs.

Table 2-15 lists the studies and corresponding HECs, HEDs, and UFs that EPA is using for risk characterization following acute, intermediate, and chronic exposure, respectively. For consistency, all HEDs are expressed as daily doses and all HECs are based on daily, continuous concentrations (24 hours/day) assuming a breathing rate for individuals at rest. Adjustments to exposure durations and frequencies and breathing rates are made in the exposure estimates used to calculate risks for individual exposure scenarios. Previous sections and systematic review supplemental files [list] provide additional information on key studies.

Hazard values were not derived for cancer, because EPA determined that the carcinogenicity of *o*-dichlorobenzene is not supported by the weight of scientific evidence and quantitative cancer risk assessment of *o*-dichlorobenzene is not necessary.

Table 2-15. Most Protective PODs for Each Exposure Scenario and Route

Target Organ System (Route)	Species	Duration Range of Co-Critical Studies[a]	Effects Observed	HEC or HED (units) [mg/m^3 if applicable]	Co-critical PODs[a]	References	Confidence in POD
Respiratory Toxicity (Inhalation)	Mice	4 days to 90 days	Nasal olfactory tissue damage, mechanism-informed superset gene expression	BMDL = 0.45 ppm[b] (2.71 mg/m^3) based on U.S. EPA (2026l); NIEHS (2025a)	0.45 ppm (UF = 30)	U.S. EPA (2026l); NIEHS (2025a)	Robust
					2.0 ppm (UF = 100)	Cho et al. (2023)	
					4.3 ppm (UF = 300)	Zissu (1995)	
Liver Toxicity (Oral/Dermal)	Mice, Rats	1 day to 90 days	Increased liver weight, serum ALT, histopathology/ mechanism-informed superset gene expression	$BMDL_{1SD}$ = 11 mg/kg-day based Umemura et al. (1996)	11.0 mg/kg-day (UF = 30)	Umemura et al. (1996)	Robust
					12.0 mg/kg-day (UF = 30)	Robinson et al. (1991)	
					8.26 mg/kg-day (UF = 30)	U.S. EPA (2026l); NIEHS (2025a)	

[a] Co-critical hazard values reflect converging evidence across studies when normalized for differences in UF. Also see Table 2-6 and Table 2-8.
[b] An 8 hour HEC will be separately calculated in the risk evaluation for use in consumer risk assessment to better align with expected exposure durations.

3 ENVIRONMENTAL HAZARD ASSESSMENT

3.1 Approach and Methodology

EPA used studies with overall quality determinations of high and medium to characterize the environmental hazards of *o*-dichlorobenzene for surrogate species representing various receptor groups, including aquatic vertebrates, aquatic invertebrates, aquatic plants and algae, terrestrial vertebrates, terrestrial invertebrates, and terrestrial plants. Hazard studies with mammalian wildlife exposed to *o*-dichlorobenzene were not available; therefore, EPA used ecologically relevant endpoints from laboratory rat and mouse studies (model organisms that are commonly used to evaluate human health hazards) to establish a hazard threshold for terrestrial vertebrates. Although two studies with overall quality determinations of high and medium contained avian hazard data for exposures to *o*-dichlorobenzene, no apical hazards (*i.e.*, growth, mortality, reproduction) were measured in those studies. Because no apical endpoints were measured in avian studies, EPA could not establish a hazard threshold for avian species.

TSCA requires that EPA use data and/or information in a manner consistent with the best available science and that the Agency base decisions on the weight of scientific evidence. To meet the TSCA science standards, EPA applies a systematic review process to identify data and information across taxonomic groups for both aquatic and terrestrial organisms with a focus on apical endpoints (*e.g.,* those affecting survival, growth, or reproduction). The data collection, data evaluation, and data integration stages of the systematic review process are used to develop the hazard assessment to support the integrative risk characterization. EPA completed the review of environmental hazard data and information sources during risk evaluation using the data quality review evaluation metrics and the rating criteria described in the 2021 *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021) and the *Draft Systematic Review Protocol for o-Dichlorobenzene* (U.S. EPA, 2026k). Studies identified and evaluated by the Agency were assigned an overall quality determination of high, medium, low, or uninformative. Study quality was evaluated based on a rubric that included consideration of the following seven overarching domains: test substance, test design, exposure characterization, test organism, outcome assessment, confounding/variable control, and data presentation/analysis. Several metrics within each of these domains were evaluated for each study, and an overall study quality determination was assigned based on the overall evaluation. Because data on toxicity of *o*-dichlorobenzene are numerous, EPA systematically evaluated all data for this hazard characterization but relied only on high- and medium-quality studies for purposes of hazard characterization. Relevance of the studies to the *o*-dichloroethane hazard and risk assessment is considered as part of the weight of scientific evidence, which is presented in subsections associated with each concentration of concern (COC) below.

A SSD analysis was used to derive an acute aquatic hazard threshold. An SSD is a type of probability distribution of toxicity values from multiple species that models the variation in sensitivity of species to a particular chemical stressor and is generated by fitting a statistical distribution function to the proportion of species affected as a function of concentration or dose. It can be used to visualize which species are most sensitive to toxic chemical exposure and to predict a concentration of a toxic chemical that is hazardous to a percentage of test species. Empirical data included in the SSD analysis were limited to LC50 (concentration which is lethal to 50% of test organisms) values at or below the limit of water solubility (141 mg *o*-dichlorobenzene/L water). Predicted acute hazard data for aquatic invertebrates and vertebrates were also generated using EPA's Web-Based Interspecies Correlation Estimation Web-ICE (v 4.0) toxicity predictions tool (U.S. EPA, 2024b). Web-ICE is a tool developed by the U.S. EPA that estimates the acute toxicity of a chemical to a species, genus, or family from

known toxicity of the chemical to a surrogate species. A deterministic approach was used to derive all other environmental hazard thresholds.

3.2 Aquatic Species

EPA reviewed studies for *o*-dichlorobenzene toxicity to aquatic organisms. Studies that received an overall quality determination of high or medium quality were used to derive hazard thresholds and are detailed in subsections below. Some studies may have included multiple endpoints, species, and test durations. Studies that received an overall quality determination of low or uninformative were reviewed and considered but not used to derive hazard thresholds.

3.2.1 Acute Toxicity of *o*-Dichlorobenzene in Aquatic Vertebrates and Invertebrates

Overall, acute toxicity of *o*-dichlorobenzene on aquatic vertebrates was observed with 96-hour LC50s for mortality ranging from 1.58 mg/L in rainbow trout (*Oncorhynchus mykiss*) to 9.47 mg/L in fathead minnow (*Pimephales promelas*) (Table 3-1). Several studies measured acute toxicity of *o*-dichlorobenzene on aquatic vertebrates at multiple time points. In rainbow trout, acute exposures to *o*-dichlorobenzene did not yield a statistical increase in LC50 with increased exposure time (24 h LC50 = 1.65 mg/L, 48 h LC50 = 1.58 mg/L, 72 h LC50 = 1.58 mg/L) (Call et al., 1983) consistent with measurements in fathead minnow (24 h LC50 ≥10.5≤15.25 mg/L, 48 h LC50 ≥10.5≤15.25 mg/L, 72 h LC50 ≥10.5≤15.25 mg/L) (Geiger et al., 1986). Loss of equilibrium, measured as inability to swim or lack of motion, was also observed following 96 hours of exposure for rainbow trout (EC50 = 1.55 mg/L; EC50 is the effect concentration at which 50% of test organisms exhibit an effect) and fathead minnows (EC50 = 4.38 mg/L (Geiger et al., 1986; Ahmad et al., 1984)) though these endpoints were not used for derivation of hazard thresholds.

Toxicity data are typically log-normally distributed with central tendencies best represented by geometric means (Klaassen, 1986). The geometric mean of all acute hazard LC50 endpoints for aquatic vertebrates across durations and species was 3.59 mg/L; the geometric mean of LC50s from 96-hour toxicity tests across aquatic vertebrate studies was 3.24 mg/L (Table 3-1). Empirical data evaluated (Table 3-1) were consistent with toxicity estimated using the Ecological Structure and Activity Relationships (ECOSAR) predictive model version 2.2 (U.S. EPA, 2022). Specifically, the 96-hour fish acute toxicity ECOSAR estimate (Chemical Class: Neutral Organics) was 6.96 mg/L LC50 (Appendix I) providing additional confidence in available data.

The bolded values in Table 3-1 and Table 3-2 describe data which were used as inputs for generating Web-ICE predictions and within a species-sensitivity distribution analysis (SSD) (Raimondo, 2010) (Appendix H). Specifically, LC50 values from 96-hour toxicity tests evaluating mortality were used as the 96-hour duration resulted in a more robust dataset and allowed for standardized and comparable measures of acute toxicity. Additionally, 96-hours is the standard duration used by Web-ICE so the empirical and predicted data align.

Table 3-1. Acute Toxicity of *o*-Dichlorobenzene in Aquatic Vertebrates

Test Organism (Species)	Hazard Values (mg/L)	Endpoint	Exposure Duration (hours)	Effect	Citation (Study Quality)
Rainbow Trout (*Oncorhynchus mykiss*)	**1.61**	LC50	96	Mortality	Ahmad et al. (1984) (Medium)
Rainbow Trout (*Oncorhynchus mykiss*)	**1.67**	LC50	96	Mortality	Dow Chemical (1974) (High)
Rainbow Trout (*Oncorhynchus mykiss*)	1.65	LC50	22	Mortality	Call et al. (1983) (Medium)
Rainbow Trout (*Oncorhynchus mykiss*)	1.58	LC50	48	Mortality	Call et al. (1983) (Medium)
Rainbow Trout (*Oncorhynchus mykiss*)	1.58	LC50	72	Mortality	Call et al. (1983) (Medium)
Rainbow Trout (*Oncorhynchus mykiss*)	**1.58**	LC50	96	Mortality	Call et al. (1983) (Medium)
Rainbow Trout (*Oncorhynchus mykiss*)	1.55	EC50	96	Behavioral; Loss of Equilibrium	Ahmad et al. (1984) (Medium)
Fathead Minnow (*Pimephales promelas*)	**9.47**	LC50	96	Mortality	Geiger et al. (1986) (High)
Fathead Minnow (*Pimephales promelas*)	**6.03**	LC50	96	Mortality	Sum et al. (1993) (High)
Fathead Minnow (*Pimephales promelas*)	4.38	EC50	96	Behavioral; Loss of Equilibrium	Geiger et al. (1986) (High)
Guppy (*Poecilia reticulata*)	**4.79**	LC50	96	Mortality	Sum et al. (1993) (High)
Bluegill (*Lepomis macrochirus*)	6.3	LC50	24	Mortality	Buccafusco et al. (1981) (Uninformative)

Test Organism (Species)	Hazard Values (mg/L)	Endpoint	Exposure Duration (hours)	Effect	Citation (Study Quality)
Bluegill (*Lepomis macrochirus*)	5.6	LC50	96	Mortality	Buccafusco et al. (1981) (Uninformative)
Sheepshead Minnow (*Cyprinodon variegatus*)	9.3	LC50	24	Mortality	Heitmuller et al. (1981) (Uninformative)
Sheepshead Minnow (*Cyprinodon variegatus*)	9.7	LC50	72	Mortality	Heitmuller et al. (1981) (Uninformative)
Sheepshead Minnow (*Cyprinodon variegatus*)	9.7	LC50	96	Mortality	Heitmuller et al. (1981) (Uninformative)
Zebra Danio (*Danio rerio*)	>180	LC50	96	Mortality	Versonnen et al. (2003) (Low)
Zebra Danio (*Danio rerio*)	6.8	LC50	24	Mortality	Calamari et al. (1983) (Uninformative)
Catla (*Gebelion catla*)	1.4	LC50	96	Mortality	Ganesan et al. (2013) (Low)
European Flounder (*Platichthys flesus*)	4.61	LC50	96	Mortality	Furay and Smith (1995) (Uninformative)
Sole (*Solea solea*)	4.19	LC50	96	Mortality	Furay and Smith (1995) (Uninformative)
Rainbow Trout (*Oncorhynchus mykiss*)	2.3	LC50	24	Mortality	Calamari et al. (1983) (Uninformative)
Fathead Minnow (*Pimephales promelas*)	76.3	LC50	48	Mortality	Curtis et al. (1979) (Uninformative)
Fathead Minnow (*Pimephales promelas*)	57.0	LC50	96	Mortality	Curtis et al. (1979) (Uninformative)

Bolded values indicate vertebrate data used to derive acute aquatic COC using SSD (96-hour durations) (Appendix H). The acute aquatic COC generated incorporated both vertebrate and invertebrate (Table 3-2) data.

Of the high and medium quality studies evaluating acute toxicity in aquatic invertebrates, several contained acute endpoints that identified definitive hazard values for *o*-dichlorobenzene that are detailed below. Overall, acute toxicity of *o*-dichlorobenzene to aquatic invertebrates ranged from 1.7 mg/L 24-hour immobilization EC50 in *Daphnia magna* (Kühn et al., 1989) to 19.9 mg/L 24-hour LC50 in midges (Call et al., 1983). Sediment aquatic invertebrates had comparable *o*-dichlorobenzene toxicity ranges relative to pelagic aquatic invertebrates. For example, midge exposure to *o*-dichlorobenzene in water yielded a 48-hour LC50 of 12.0 mg/L (Call et al., 1983) though the 48-hour LC50 for amphipods exposure in water was two orders of magnitude lower (0.78 mg/L) (Tong et al., 2010).

Toxicity data are typically log-normally distributed with central tendencies best represented by geometric means (Klaassen, 1986). The geometric mean of all acute hazard endpoints for aquatic invertebrates across duration and species was 3.05 mg/L (Table 3-2). The empirical data evaluated (Table 3-2) were consistent with estimated toxicity using the Ecological Structure and Activity Relationships (ECOSAR) predictive model version 2.2 (U.S. EPA, 2022). Specifically, the 48-hour daphnid acute toxicity ECOSAR estimate (Chemical Class: Neutral Organics) was 4.49 mg/L LC50 (Appendix I) providing additional confidence in available data.

The bolded values in Table 3-2 indicate data which were used as inputs for generating Web-ICE predictions and within a species-sensitivity distribution analysis (SSD) (Appendix H). Specifically, LC50 values from 96-hour toxicity tests evaluating mortality were used in generating Web-ICE predictions and SSD analyses as this duration provided a more robust dataset and allowed for standardized and comparable measures of acute toxicity. Additionally, 96-hours is the standard duration used by Web-ICE so the empirical and predicted data align.

Table 3-2. Acute Toxicity of *o*-Dichlorobenzene in Aquatic Invertebrates

Test Organism (Species)	Habitat	Hazard Values (mg/L)	Endpoint	Duration (hours)	Effect	Citation (Study Quality)
Water Flea (*Daphnia magna*)	Pelagic	1.70	EC50	24	Immobilization	Kühn et al. (1989) (Medium)
Copepod, eggs (*Eurytemora affinis*)	Epibenthic	3.5 **15.9**	EC50 LC50	96	Mortality (Hatching)	Lindley et al. (1999) (Medium)
Copepod, eggs (*Acartia bifilosa*)	Pelagic	14.2 % saturation	LC50	96	Mortality (Hatching)	Lindley et al. (1999) (Medium)
Copepod, eggs (*Acartia clausi*)	Pelagic	**2.1**	LC50	96	Mortality (Hatching)	Lindley et al. (1999) (Medium)
Sea Urchin (*Paracentrotus lividus*)	Benthic	1.5	LOEC	From zygote to pluteus larval stage	Reproduction	(Pagano et al., 1988) (Medium)

Test Organism (Species)	Habitat	Hazard Values (mg/L)	Endpoint	Duration (hours)	Effect	Citation (Study Quality)
Sea Urchin (*Paracentrotus lividus*)	Benthic	15	NOEC	48	Development/growth	(Pagano et al., 1988) (Medium)
Amphipod (*Melita longidactyla*)	Benthic	0.78	LC50	48	Mortality	Tong et al. (2010) (Medium)
Midge (*Tanytarsus dissimilis*)	Benthic	19.9	LC50	24	Mortality	Call et al. (1983) (Medium)
Midge (*Tanytarsus dissimilis*)	Benthic	12.0	LC50	48	Mortality	Call et al. (1983) (Medium)
Protozoa (*Tetrahymena pyriformis*)	Pelagic	51.0	EC50	24	Development/growth	Yoshioka et al. (1985) (Low)
Daggerblade Grass Shrimp (*Palaemon pugio*)	Benthic	10.0	LC50	96	Mortality	Curtis and Ward (1981) (Low)
Water Flea (*Daphnia magna*)	Pelagic	0.78	IC50	24	Immobilization	Calamari et al. (1983) (Uninformative)
Water Flea (*Daphnia magna*)	Pelagic	7.7	IC50	48	Immobilization	Deneer et al. (1988) (Low)
Water Flea (*Daphnia magna*)	Pelagic	7.8	IC50	48	Immobilization	Deneer et al. (1988) (Low)
Water Flea (*Daphnia magna*)	Pelagic	5.7	IC50	48	Immobilization	Deneer et al. (1988) (Low)
Water Flea (*Daphnia magna*)	Pelagic	3.3	IC50	48	Immobilization	Deneer et al. (1988) (Low)
Water Flea (*Daphnia magna*)	Pelagic	6.6	IC50	48	Immobilization	Deneer et al. (1988) (Low)
Water Flea (*Daphnia magna*)	Pelagic	8.8	IC50	48	Immobilization	Deneer et al. (1988) (Low)

Test Organism (Species)	Habitat	Hazard Values (mg/L)	Endpoint	Duration (hours)	Effect	Citation (Study Quality)
Water Flea (*Daphnia magna*)	Pelagic	7.3	IC50	48	Immobilization	Deneer et al. (1988) (Low)
Water Flea (*Daphnia magna*)	Pelagic	3.8	EC50	48	Immobilization	Rose et al. (1998) (Uninformative)
Water Flea (*Daphnia magna*)	Pelagic	2.4E−06	LC50	48	Mortality	Abernethy et al. (1986) (Uninformative)
Water Flea (*Ceriodaphnia dubia*)	Pelagic	0.70	EC50	48	Immobilization	Rose et al. (1998) (Uninformative)
Brine Shrimp (*Artemia salina*)	Benthic	1.5E−05	LC50	24	Mortality	Abernethy et al. (1986) (Uninformative)
Daggerblade Grass Shrimp (*Palaemon pugio*)	Benthic	14.3	LC50	24	Mortality	Curtis et al. (1979) (Uninformative)
Daggerblade Grass Shrimp (*Palaemon pugio*)	Benthic	10.3	LC50	48	Mortality	Curtis et al. (1979) (Uninformative)
Daggerblade Grass Shrimp (*Palaemon pugio*)	Benthic	9.4	LC50	96	Mortality	Curtis et al. (1979) (Uninformative)

Bolded values indicate invertebrate data used to derive acute aquatic COC using SSD (96-hour LC50) selected to standardize durations (Appendix H). The acute aquatic COC generated incorporated both invertebrate and vertebrate (Table 3-1) data.

3.2.1.1 Acute Aquatic Concentration of Concern (COC)

EPA used an SSD to derive the acute aquatic COC for *o*-dichlorobenzene. The hazardous concentration is represented as an HC_p, where p is the percent of species below the threshold. EPA used an HC_{05} (a Hazardous Concentration threshold for 5% of species) to estimate a concentration that would protect 95% of species. This HC_{05} can then be used to derive a COC, which is the estimated hazardous concentration of *o*-dichlorobenzene in water for aquatic organisms. For the probabilistic approach used for acute aquatic hazard, the lower bound of the 95% CI of the HC_{05} was used to account for uncertainty. The application of ICE models eliminates the need for assessment factors (AFs) by extrapolating toxicity to a diversity of species representing a wide range of aquatic taxa with surrogate species sensitivity (Awkerman et al., 2014). Raimondo et al (2025) found that using the lower 95% CI as the COC reduced aleatory uncertainty. EPA has more confidence in probabilistic approaches (relative to deterministic approaches) when enough data are available because an HC_{05} is representative of a larger

portion of species in the environment. Generally, EPA considers the probabilistic approach for aquatic hazard (*i.e.,* an SSD) appropriate when hazard values for at least eight species are represented in the data set.

The acute COC for aquatic organisms (vertebrates and invertebrates combined) was derived from an SSD that contained 96-hour LC50 empirical data for five species identified in systematic review (bolded values in Table 3-1 and Table 3-2). This duration provided a more robust dataset and is also the standard duration used by Web-ICE so the empirical and predicted data align. Initial analyses were also bolstered by additional predicted EC50 and LC50 values from the Web-ICE v4.0 toxicity value estimation tool. Web-ICE was used to obtain estimated acute toxicity values for *o*-dichlorobenzene in species that were not represented in the empirical data set. After reviewing the possible statistical distributions for the SSD, the Metropolis-Hastings method was chosen with a Logistic distribution. This choice was based on an examination of p-values for goodness of fit, visual examination of Q-Q plots, posterior diagnostics, and evaluation of the line of best fit near the low-end of the SSD. The HC_{05} for this distribution is 0.5956 mg/L *o*-dichlorobenzene (Figure 3-1). After taking the lower 5th percentile of this HC_{05} as an alternative to the use of AFs, the acute aquatic COC for vertebrates and invertebrates is 0.3799 mg/L *o*-dichlorobenzene. See Appendix H for details of the SSD that was used to derive the acute aquatic COC for *o*-dichlorobenzene.

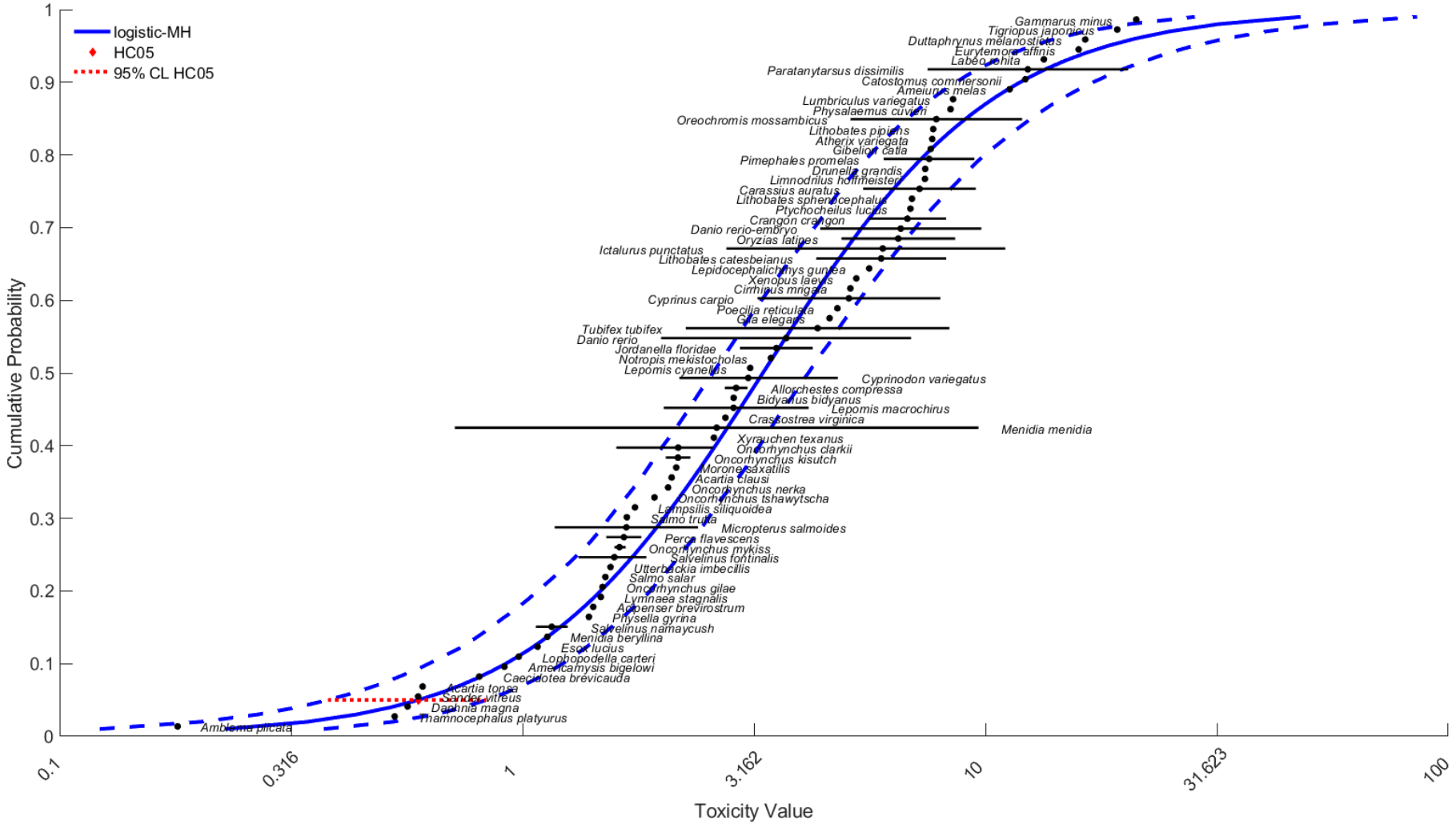

Figure 3-1. Species Sensitivity Distribution (SSD) of Acute Hazard Effects of *o*-Dichlorobenzene to Aquatic Organisms (HC_{05} lower CI = 0.3799 mg/L)

Best model fit depicted with Metropolis-Hastings method and Logistic distribution.

3.2.1.2 Weight of Scientific Evidence for Acute Aquatic Concentration of Concern (COC)

EPA uses several considerations when weighing and weighing scientific evidence to determine confidence in the environmental hazard data. These considerations include the quality of the database, consistency, strength and precision, biological gradient, and relevance. This approach is described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021). Table_Apx G-1 summarizes how these considerations were determined for each environmental hazard threshold with additional criteria for assessing confidence is described in Appendix G.

The weight of evidence suggests that *o*-dichlorobenzene poses acute hazard effects to vertebrate and invertebrate aquatic organisms at 0.38 mg/L for *o*-dichlorobenzene. EPA has robust confidence in this hazard threshold because the quality of the database of studies included high or medium quality studies across a range of species that consistently resulted in LC50s between 1.58 and 19.9 mg/L *o*-dichlorobenzene. These studies were conducted with reasonable designs and results, which enabled precise LC50 calculations (Table 3-1 and Table 3-2) and were consistent with ECOSAR predictions.

EPA used a probabilistic technique (SSD) to derive a COC (Appendix H) that is protective of 95% of the aquatic animals in a community by incorporating hazard values across species and habitats. EPA has robust confidence in the SSD with empirical data across species and phyla that inhabit both freshwater and marine environments. Limitations of an SSD include its reliance on model species that may not exist or interact in the same ecological community and are weighted equally. Also, the shape of the data distribution that is fitted to the effects data can be subjective and dependent on the three or four lowest values (Newman et al., 2000). The use of Interspecies Correlation Estimation (ICE) models (*e.g.,* EPA Web-ICE) was used to add surrogate LC50 data from additional species to SSD distributions. However, adding modeled data also adds additional variation and epistemic uncertainty. Notwithstanding the limitations of SSD analyses (*e.g.*, reliance on model species that may not be representative, limited model selection), this method is widely used and accepted in risk assessments (Raimondo, 2010). EPA has robust confidence in the quality, consistency, strength and precision, and relevance of the studies used in determining the acute aquatic COC (0.38 mg *o*-dichlorobenzene/L water).

3.2.2 Chronic Toxicity of *o*-Dichlorobenzene in Aquatic Vertebrates and Invertebrates

Chronic toxicity of *o*-dichlorobenzene on aquatic vertebrates ranged from above a NOAEC of 1 mg/L with 14 days of exposure to zebrafish to 100% embryo mortality at 47.5 mg/L with 5 days of exposure to leopard frog embryos. Toxicity of *o*-dichlorobenzene on developmental stages of rainbow trout and amphibians found trout were more sensitive than amphibians (Black et al., 1982). Specifically, the trout 23-day LC50 was 3.01 mg/L; whereas the amphibian 9-day LC50 was 5.56 mg/L though exposure durations differed. Both trout and amphibian hatchability of embryo-larval stages was reduced with increasing *o*-dichlorobenzene concentrations (84% hatchability at 2.31 mg/L and 61% hatchability at 12.3 mg/L, respectively) (Black et al., 1982). The geometric mean of chronic hazard LC50 values for aquatic vertebrates across all organisms and durations is 3.9 mg/L. Though empirical studies on the chronic toxicity of *o*-dichlorobenzene to aquatic vertebrates are limited (Table 3-3), measured toxicity data are higher than estimated toxicity using the ECOSAR predictive model version 2.2 (U.S. EPA, 2022). Specifically, the predicted chronic value (ChV) for fish from ECOSAR estimate (Chemical Class: Neutral Organics) was 0.791 mg/L (Appendix I) providing additional confidence in the available empirical data.

Table 3-3. Chronic Toxicity of *o*-Dichlorobenzene in Aquatic Vertebrates

Test Organism (Species)	Hazard Values (mg/L)	Endpoint	Exposure Duration (days)	Effect	Citation (Study Quality)
Rainbow Trout, embryo (*Oncorhynchus mykiss*)	**3.01**	LC50	23	Mortality; Hatching Success	Black et al. (1982) (High)
Rainbow Trout, embryo (*Oncorhynchus mykiss*)	**3.01**	LC50	27	Mortality; Hatching Success	Black et al. (1982) (High)
Rainbow Trout, embryo (*Oncorhynchus mykiss*)	13.2	100% Mortality	27	Mortality; Hatching Success	Black et al. (1982) (High)
Rainbow Trout, embryo (*Oncorhynchus mykiss*)	**1.54**	LC50	6	Mortality	Call et al. (1983) (Medium)
Leopard Frog, embryo (*Lithobates pipiens*)	**12.07**	LC50	5	Mortality; Hatching Success	Black et al. (1982) (High)
Leopard Frog, embryo (*Lithobates pipiens*)	**5.56**	LC50	9	Mortality; Hatching Success	Black et al. (1982) (High)
Leopard Frog, embryo (*Lithobates pipiens*)	47.5	100% Mortality	5	Mortality; Hatching Success	Black et al. (1982) (High)
Zebrafish (*Danio rerio*)	1.00	NOAEC	14	Development/Growth	(Versonnen et al., 2003) (Medium)
Zebrafish (*Danio rerio*)	1.00	NOAEC	14	Reproductive/Teratogenic	(Versonnen et al., 2003) (Medium)
Catla (*Gibelion catla*)	0.18	LOAEC	28	Genotoxicity	(Ganesan et al., 2013) (Medium)
Bold values were used to calculate the geometric mean of chronic hazard LC50 values.					

Chronic toxicity of *o*-dichlorobenzene exposure to aquatic invertebrates ranged from 0.02 mg/L LOAEC with sixty days of exposure to amphipods (Tong et al., 2010) to effects on reproduction at 5.4 mg/kg sediment with ten days of exposure to sediment midge (Eurofins Eag Agroscience, 2024) (Table 3-4). Two environmental hazard toxicity tests were completed in conjunction with a Test Order under the authority in TSCA section 4(a)(2) to evaluate chronic toxicity of *o*-dichlorobenzene to sediment invertebrates. In the life-cycle toxicity test with midge (*Chironomus dilutus*) using spiked sediment assays, measured NOAEC and LOAEC were greater than 199 mg/kg sediment based on mean measured

concentrations in the sediment normalized for organic carbon for survival endpoints as well as for growth, emergence, and reproduction endpoints (Eurofins Eag Agroscience, 2025). After 16 days of exposure, mean survival ranged from 97% in control groups to 98 and 100% with exposure to 1.4 and 4.0 mg/kg treatment concentrations, respectively. In the 10-day static toxicity test using spiked sediment, sediment midge (*Chironomus dilutus*) survival was not influenced at experimental treatment concentrations (maximum = 24 mg/kg sediment) though decreases in growth (as measured by biomass) occurred at treatment concentrations 5.4 mg/kg sediment and above (Eurofins Eag Agroscience, 2024). In a long-term (60 days) study of the effects of *o*-dichlorobenzene on amphipod reproduction, progeny production declined with exposure duration (Tong et al., 2010). Specifically, at 21 days of exposure, there was a 57% reduction in females producing progeny at 0.020 mg/L *o*-dichlorobenzene exposure relative to controls and a 64% reduction in progeny produced at 60 days of exposure (Tong et al., 2010).

Though empirical studies on the chronic toxicity of *o*-dichlorobenzene to aquatic invertebrates are limited, measured toxicity data were generally higher than toxicity estimates using the ECOSAR predictive model version 2.2 (U.S. EPA, 2022). Specifically, the ChV for Daphnids exposed to *o*-dichlorobenzene estimated by ECOSAR (Chemical Class: Neutral Organics) was 0.624 mg/L (Appendix I) providing additional confidence in available data.

Table 3-4. Chronic Toxicity of *o*-Dichlorobenzene in Aquatic Invertebrates

Test Organism (Species)	Habitat	Hazard Values	Endpoint	Duration (days)	Effect	Citation (Study Quality)
Water Flea (*Daphnia magna*)	Pelagic	0.63 mg/L	NOAEC	21	Mortality	Kühn et al. (1989) (Medium)
Midge (*Chironomus dilutus*)	Benthic	>199 mg/kg >199 mg/kg	LOAEC NOAEC	16	Mortality	Eurofins Eag Agroscience (2025) (High)
Midge (*Chironomus dilutus*)	Benthic	>199 mg/kg 199 mg/kg	LOAEC NOAEC	50	Mortality	Eurofins Eag Agroscience (2025) (High)
Midge (*Chironomus dilutus*)	Benthic	>24 mg/kg	LC50	10	Mortality	Eurofins Eag Agroscience (2024) (High)
Midge (*Chironomus dilutus*)	Benthic	5.4 mg/kg 2.2 mg/kg	LOAEC NOAEC	10	Development/ Growth	Eurofins Eag Agroscience (2024) (High)
Water Flea (*Daphnia magna*)	Pelagic	0.63 mg/L	NOAEC	21	Reproduction	Kühn et al. (1989) (Medium)
Amphipod (*Melita longidactyla*)	Benthic	**2.0E−02 mg/L** 5.0E−03 mg/L	LOAEC NOAEC	21	Reproduction	Tong et al. (2010) (Medium)
Water Flea (*Daphnia magna*)	Pelagic	0.55 mg/L	EC50	14	Reproduction	Calamari et al. (1983) (Low)

Test Organism (Species)	Habitat	Hazard Values	Endpoint	Duration (days)	Effect	Citation (Study Quality)
Bold value was used to calculate the chronic aquatic concentration of concern (COC). Data from (Eurofins Eag Agroscience, 2024) and (Eurofins Eag Agroscience, 2025) are in conjunction with a Test Order under the authority in TSCA section 4(a)(2) to evaluate chronic toxicity of *o*-dichlorobenzene to sediment invertebrates.						

3.2.2.1 Chronic Aquatic Concentration of Concern (COC)

Though empirical studies were limited to assess the effects of chronic *o*-dichlorobenzene on aquatic vertebrates and invertebrates, the most sensitive endpoint for vertebrates was associated with 6-day *o*-dichlorobenzene exposure to rainbow trout embryos yielding 50% mortality (LC50) at 1.54 mg/L (Call et al., 1983) and the most sensitive endpoint for invertebrates was associated with 21-day *o*-dichlorobenzene exposure to amphipods yielding a LOAEC on reproduction at 0.020 mg/L (Tong et al., 2010). The most sensitive toxicity endpoint is used to establish a conservative effects threshold protective of sensitive species.

An AF of 10 was applied to account for species variability and non-lethal effects as well as scientific uncertainty. This incorporates the uncertainty in the actual threshold dose, which may have been lower than the lowest dose studied. After applying an AF of 10, the chronic COC for aquatic vertebrates for *o*-dichlorobenzene is 0.15 mg/L. After applying an AF of 10, the chronic COC for aquatic invertebrates is 0.002 mg/L *o*-dichlorobenzene.

3.2.2.2 Weight of Scientific Evidence for Chronic Aquatic Concentration of Concern (COC)

The weight of evidence suggests that *o*-dichlorobenzene poses chronic hazard effects to vertebrate animals at 0.15 mg/L *o*-dichlorobenzene and chronic hazard effects to invertebrate animals at 0.002 mg/L *o*-dichlorobenzene. EPA has moderate confidence in the weight of evidence for toxicity to aquatic invertebrates and vertebrates with chronic *o*-dichlorobenzene exposure due to the limited number of species evaluated though studies available were of high and medium quality and measured toxicity endpoints ranging only two orders of magnitude across estimates (0.55 to 47.5 mg/L *o*-dichlorobenzene; Table 3-3, Table 3-4) consistent with ECOSAR predictions. Table_Apx G-1 summarizes how these considerations were determined for each environmental hazard threshold with additional criteria for assessing confidence described in Appendix F.

3.2.3 Toxicity of *o*-Dichlorobenzene in Aquatic Plants and Algae

Toxicity of *o*-dichlorobenzene on aquatic plants and algae ranged from 2.2 mg/L EC50 with 96 hours of exposure to green algae to 23.3 mg/L EC50 with 48 h exposure to diatoms (Table 3-5). The effect of *o*-dichlorobenzene on diatom growth was measured by DNA reduction which may have elevated results relative to estimates of growth as cell counts or biomass (Figueroa and Simmons, 1991) given genotypic effects (*i.e.*, DNA) may precede, or not be realized as, phenotypic effects (*i.e.*, cell size and number). Cell multiplication tests in *Scenedesmus subspicatus* green algae with exposure to *o*-dichlorobenzene yielded effective concentrations of 14 mg/L (48 h EC50) (Kühn and Pattard, 1990). Toxicity of *o*-dichlorobenzene to freshwater algae was comparable to toxicity measurements in marine algae.

The geometric mean of all hazard endpoints for aquatic plants and algae across species and durations is 10.8 mg/L. Empirical data evaluated (Table 3-5) were consistently higher than estimated toxicity using the Ecological Structure and Activity Relationships (ECOSAR) predictive model version 2.2 (U.S. EPA, 2022). Specifically, the green algae EC50 with 96-hour exposure was 5.66 mg/L and the green algae chronic toxicity value (ChV) ECOSAR estimate (Chemical Class: Neutral Organics) was 1.97 mg/L (Appendix I).

Table 3-5. Toxicity of *o*-Dichlorobenzene in Aquatic Plants and Algae

Test Organism (Species)	Hazard Values (mg/L)	Endpoint	Duration (hours)	Effect	Citation (Study Quality)
Green algae (*Selenastrum capricornutum*)	**2.2**	EC50	96	Growth	Galassi and Vighi (1981) (Medium)
Green Algae (*Scenedesmus subspicatus*)	14	EC50	48	Cell multiplication	Kühn and Pattard (1990) (High)
Diatom (*Cyclotella meneghiniana*)	23.3	EC50	48	DNA reduction	Figueroa and Simmons (1991) (High)
Green flagellate (*Pyramimonas* sp.)	17.9	EC50	72	Growth inhibition	Ma et al. (1997) (Medium)
Green flagellate (*Platymonas subcordiformis*)	16.9	EC50	72	Growth inhibition	Ma et al. (1997) (Medium)
Yellow green algae (*Nannochloropsis oculata*)	13.1	EC50	72	Growth inhibition	Ma et al. (1997) (Medium)
Marine algae (*Chlorella marina*)	16.2	EC50	72	Growth inhibition	Ma et al. (1997) (Medium)
Diatom (*Phaeodactylum tricornutum*)	12.8	EC50	72	Growth inhibition	Ma et al. (1997) (Medium)
Green Algae (*Selenastrum capricornutum*)	10	EC50	3	Photosynthesis inhibition	Calamari et al. (1983) (Uninformative)
Bold value was used to calculate the aquatic plant and algae concentration of concern (COC).					

3.2.3.1 Aquatic Plant and Algae Concentration of Concern (COC)

The most sensitive endpoint evaluating toxicity of *o*-dichlorobenzene to aquatic plants and algae was with 96-hour exposure to green algae yielding an EC50 of 2.2 mg/L (Galassi and Vighi, 1981). The most sensitive toxicity endpoint is used to establish a conservative effects threshold protective of sensitive species. An AF of 10 was applied to account for species variability and incorporates the uncertainty in the actual threshold dose, which may have been lower than the lowest dose studied. After applying an AF of 10, the COC for aquatic plants and algae is 0.22 mg/L.

3.2.3.2 Weight of Scientific Evidence for Aquatic Plant and Algae Concentration of Concern (COC)

The weight of evidence suggests that *o*-dichlorobenzene poses effects on aquatic plants and algae at 0.22 mg/L. EPA has moderate confidence in the weight of evidence for toxicity to aquatic plants and algae due to the limited number of species evaluated though studies available were of high and medium quality and measured toxicity estimates were within the same magnitude across estimates (10 to 23.3 mg/L *o*-dichlorobenzene; Table 3-5) as well as consistent with ECOSAR predictions. Table_Apx G-1

summarizes how these considerations were determined for each environmental hazard threshold with additional criteria for assessing confidence described in Appendix F.

3.3 Terrestrial Species

o-Dichlorobenzene will volatilize from soil surfaces and is not expected to sorb to soils or organic matter based on its physical and chemical properties (U.S. EPA, 2026c). The predominant environmental releases of *o*-dichlorobenzene to land are disposal via underground injection or landfills (U.S. EPA, 2026i). Therefore, there are no appreciable direct releases to land. Further, modeled air deposition of *o*-dichlorobenzene to land is negligible and available monitoring data indicate *o*-dichlorobenzene is rarely detected in groundwater. Considering these lines of evidence, *o*-dichlorobenzene is not expected to be present or persist on land and result in terrestrial animal exposure. Environmental fate and transport data also indicate *o*-dichlorobenzene does not bioaccumulate (U.S. EPA, 2026i). Thus, there is no dietary exposure of *o*-dichlorobenzene to terrestrial organisms EPA reviewed studies for *o*-dichlorobenzene toxicity to terrestrial organisms via ambient air exposure.

3.3.1 Toxicity of *o*-Dichlorobenzene in Terrestrial Vertebrates

No reasonably available information was identified for exposures of *o*-dichlorobenzene to mammalian wildlife. EPA reviewed studies for toxicity to *o*-dichlorobenzene in human health animal model rodent studies that contained ecologically relevant apical endpoints (*i.e.*, reproduction, growth, mortality) with inhalation exposure. *o*-Dichlorobenzene will volatilize from soil surfaces and is not expected to sorb to soils or organic matter based on its physical and chemical properties (U.S. EPA, 2026c). Further, modeled air deposition of *o*-dichlorobenzene to land is negligible and *o*-dichlorobenzene does not bioaccumulate. Thus, the primary pathway of exposure to *o*-dichlorobenzene for terrestrial animals is via ambient air (U.S. EPA, 2026i).

Intermediate exposure (10-day) to *o*-dichlorobenzene in air yielded decreased maternal body weight gain in rats and rabbits exposed for six hours per day (Hayes et al., 1985). In male and female albino rats exposed to *o*-dichlorobenzene in air for seven hours per day, five days per week over approximately seven months, statistically significant decreases in body weight were measured in males (8.9% lower than controls) at 93 ppm (559.2 mg/m^3) exposure in air though no body weight changes were measured at that concentration in female rats, guinea pigs or rabbits (Hollingsworth et al., 1958). In a multi-generational study on the effects of *o*-dichlorobenzene inhalation on CD rats at 50, 150, and 400 ppm in chamber experiments, there were no effects on mortality or reproduction; however, mean weight gain was significantly lower in treatment groups relative to controls at the highest exposure level (400 ppm) (Schroeder and Daly, 1989).

Table 3-6. Toxicity of *o*-Dichlorobenzene to Terrestrial Vertebrates via Inhalation Exposure

Test Organism (Type)	Hazard Values	Endpoint	Duration	Effect	Citation (Study Quality)
Rat, female (Fischer-344)	400 ppm (2,400 mg/m^3)	NOAEL	10 days	Reproduction	Hayes et al. (1985) (High)
Rabbit, female (New Zealand)	400 ppm (2,400 mg/m^3)	NOAEL	10 days	Reproduction	Hayes et al. (1985) (High)
Rat, male (Albino)	49 ppm (294.6 mg/m^3) **93 ppm (559.2 mg/m^3)**	NOAEL LOAEL	6 months	Body weight	Hollingsworth et al. (1958) (Medium)

Test Organism (Type)	Hazard Values	Endpoint	Duration	Effect	Citation (Study Quality)
Mouse, female (Not specified)	49 ppm (294.6 mg/m^3)	NOAEL	6 months	Body weight	Hollingsworth et al. (1958) (Medium)
Guinea pig (Albino)	93 ppm (559.2 mg/m^3)	NOAEL	6 months	Body weight	Hollingsworth et al. (1958) (Medium)
Rabbit (Albino)	93 ppm (559.2 mg/m^3)	NOAEL	6 months	Body weight	Hollingsworth et al. (1958) (Medium)
Bold value used as the terrestrial hazard threshold.					

3.3.1.1 Terrestrial Vertebrate Hazard Value

Terrestrial organisms may be exposed to *o*-dichlorobenzene via ambient air. Based on laboratory animal model toxicity studies, toxicity of *o*-dichlorobenzene to mammals via inhalation ranged from 93 ppm (559.2 mg/m^3) in rats with 6 months exposure yielding changes in body weight to 400 ppm (2,400 mg/m^3) with 10 days of exposure yielding changes in body weight and effects on reproduction. The most sensitive LOAEL (559.2 mg/m^3) was used as the hazard threshold value for terrestrial vertebrate exposure to *o*-dichlorobenzene in air via inhalation. The most sensitive toxicity endpoint is used to establish a conservative effects threshold protective of sensitive species. EPA could not establish a definitive hazard threshold for avian species as there were no studies reasonably available.

3.3.1.2 Weight of Scientific Evidence for Terrestrial Vertebrate Hazard Value

No studies on terrestrial wildlife involving mammals were identified. In lieu of terrestrial wildlife studies, references for mammal studies as human health model organisms were used to determine a lowest and most conservative *o*-dichlorobenzene concentration that affected apical endpoints (*i.e.*, survival, reproduction, growth) and that could serve as an indication of hazard effects in wild mammal populations.

The weight of evidence suggests that *o*-dichlorobenzene poses inhalation exposure hazard effects to terrestrial mammals at 93 ppm (559.2 mg/m^3). EPA has moderate confidence in this hazard threshold given the limited availability of data, the lack of wildlife specific data, and the range of reported toxicity values. Studies were conducted with reasonable designs and results, which enabled precise estimation of effect concentrations. However, population level effects were not observed in ecologically relevant species. Considerable uncertainties surround whether or how these effects on individual growth and reproductive development translate into effects on wild mammal fitness and population parameters. Because of these uncertainties of extrapolations to wildlife mammal species, EPA has moderate confidence that the hazards are representative of the range of wild mammal species. Table_Apx G-1 summarizes how these considerations were determined for each environmental hazard threshold with additional criteria for assessing confidence described in Appendix F.

3.3.2 Toxicity of *o*-Dichlorobenzene in Terrestrial Invertebrates

EPA identified one study that received an overall quality determination of high for environmental hazard with terrestrial invertebrates exposed to *o*-dichlorobenzene. A 48-hour contact exposure of earthworms to *o*-dichlorobenzene applied to filter paper in a sealed vial resulted in a mortality LC50 of 21 µg/cm^2 (Neuhauser et al., 1985). Estimated toxicity using the Ecological Structure and Activity Relationships (ECOSAR) predictive model version 2.2 (Chemical Class: Neutral Organics) (U.S. EPA, 2022) was 184 mg/L LC50 for earthworms exposed to *o*-dichlorobenzene over 14 days (Appendix I). However, this

estimated toxicity value exceeds *o*-dichlorobenzene solubility (141 mg/L) and no effects at saturation are expected based on predictive modeling.

3.3.2.1 Terrestrial Invertebrate Hazard Value

No reasonably available studies for the effects of *o*-dichlorobenzene on terrestrial invertebrates were identified with relevant environmental exposures; the one study identified evaluating earthworm exposure to *o*-dichlorobenzene did not incorporate a relevant environmental exposure (Neuhauser et al., 1985). However, predicted toxicity to earthworms using ECOSAR indicates that toxicity is likely above solubility limits of *o*-dichlorobenzene (141 mg/L) suggesting *o*-dichlorobenzene is not bioavailable or toxic to terrestrial invertebrates at environmentally relevant concentrations.

3.3.2.2 Weight of Scientific Evidence for Terrestrial Invertebrate Hazard Value

The weight of evidence suggests that *o*-dichlorobenzene does not pose hazardous effects on terrestrial invertebrates. EPA has slight confidence in the weight of evidence for toxicity to terrestrial invertebrates due to the limited studies available. Table_Apx G-1 summarizes how these considerations were determined for each environmental hazard threshold with additional criteria for assessing confidence described in Appendix F.

3.3.3 Toxicity of *o*-Dichlorobenzene in Terrestrial Plants

There are two studies measuring the effects of *o*-dichlorobenzene exposure on terrestrial plants though these studies do not report hazard values associated with apical endpoints (*i.e.*, mortality, growth, reproduction). In air-soil-plant microcosm experiments, *o*-dichlorobenzene is predominantly lost via volatilization though some plant uptake from soil occurs (as measured by ^{14}C uptake in plant roots and shoots) which can affect plant growth (Wilson, 2003) though changes in growth were not reported. In static exposure experiments examining the transfer of *o*-dichlorobenzene through the water, leaf, and stems of wetland plants (*Myriophyllum spicatum, Bacopa caroliniana, Hydrilla verticillata*) steady state models of tissue burden occurs between 1- and 3-days following exposure (Wolf et al., 1991) suggesting limited chronic effects of *o*-dichlorobenzene on plants.

3.3.3.1 Terrestrial Plant Hazard Value

No reasonably available studies for the effects of *o*-dichlorobenzene on terrestrial plants were identified that quantified effects on apical endpoints (*i.e.*, growth, reproduction, mortality). Because no apical hazards were observed in any terrestrial plant studies, EPA did not establish a hazard threshold for terrestrial plants.

3.4 Environmental Hazard Conclusions

EPA determined that *o*-dichlorobenzene poses hazards from acute and chronic exposures to aquatic vertebrates, aquatic invertebrates, and aquatic plants and algae. After calculating the hazard thresholds that were carried forward to characterize risk, a table describing the weight of scientific evidence and uncertainties was completed to support EPA's decisions (Table 3-7). Overall, EPA has robust confidence in the weight of evidence for toxicity to aquatic vertebrates with acute *o*-dichlorobenzene exposure given the consistency in measured values documented across a range of species. EPA has moderate confidence in the weight of evidence for toxicity to aquatic invertebrates and plants as well as toxicity of *o*-dichlorobenzene to aquatic vertebrates with chronic exposure due to the limited number of species evaluated. There is also moderate confidence in the weight of evidence for toxicity of *o*-dichlorobenzene to terrestrial vertebrates as there are no studies on wildlife species and available assessments on mice and rats may not be representative. EPA has slight confidence in the weight of evidence for toxicity of *o*-dichlorobenzene to terrestrial invertebrates and plants as limited data are available. See Appendix G for more detail on how EPA weighed the scientific evidence.

3097 **Table 3-7. Environmental Hazard Thresholds for *o*-Dichlorobenzene[a]**

Receptor Group	Exposure Duration	Hazard Threshold (COC or HV)	Confidence[b]	Source
Aquatic Vertebrates	Acute	0.38 mg/L	Robust	SSD
	Chronic	0.15 mg/L	Moderate	Call et al. (1983) (Medium)
Aquatic Invertebrates	Acute	0.38 mg/L	Robust	SSD
	Chronic	0.002 mg/L	Moderate	(Tong et al., 2010) (Medium)
Aquatic Plants and Algae	NA	0.22 mg/L	Moderate	Galassi and Vighi (1981) (Medium)
Terrestrial Vertebrates	NA	93 ppm (559.2 mg/m^3)	Moderate	Hollingsworth et al. (1958) (Medium)
Terrestrial Invertebrates	NA	184 mg/L (above solubility 141 mg/L)	Slight	ECOSAR

[a] Acute aquatic hazard thresholds were determined with probabilistic modeling (SSD); all other hazard thresholds were deterministic as the lowest effect value
NA = not applicable; COC = concentration of concern; HV = hazard value; SSD = Species Sensitivity Distribution
[b] See Appendix G for additional detail on the weight of scientific evidence.

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Appendix A BENCHMARK DOSE MODELING RESULTS FOR CRITICAL ENDPOINTS

EPA performed benchmark dose (BMD) modeling using EPA's BMD modeling software (BMDS Version BMDS Online 25.1)[5] for selected critical non-cancer human health outcomes identified in the *ortho*-dichlorobenzene non-cancer hazard assessment (Section 2.3). EPA conducted BMD modeling in a manner consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMDS-generated MS Word reports for the datasets discussed below are provided in the BMDS output supplemental file (U.S. EPA, 2026b). The supplemental file includes the input data, BMDS results, and plots of the selected models.

If applicable, non-cancer endpoints selected for modeling were based on either dichotomous or continuous data. The default model selection settings in BMDS were used for all BMD modeling runs, unless otherwise stated. For dichotomous data, the default model settings include the restricted Dichotomous Hill, Gamma, Log Logistic, Multistage, and Weibull models and the unrestricted Logistic, Log Probit, Probit, and Quantal Linear models. For continuous data, the default model settings include the restricted Exponential, Hill, Polynomial, and Power models and the unrestricted Linear model.

Dichotomous models were fit employing a BMR of 10% extra risk (ER). Adequacy of model fit was judged based on the chi-square goodness-of-fit p-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit.

Continuous models were fit employing a BMR of 1 standard deviation (SD) and 10% relative deviation (RD) where appropriate. An adequate fit was judged based on the chi-square goodness-of-fit p-value (Test 4; $p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (*i.e.*, Test 2; p-value > 0.05), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected (p-value < 0.05), the dataset was run again while modeling the variance as a power function of the mean to account for nonconstant variance. If this nonconstant variance model also did not adequately fit the data (*i.e.*, Test 3; p-value < 0.05), the dataset was considered unsuitable for BMD modeling.

As applicable for both dichotomous and continuous data, the lowest BMD lower confidence level (BMDL) from all models providing adequate fit was selected if the BMDLs estimated from different models varied > 3-fold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected.

Administered concentrations/doses were converted to HECs/HEDs prior to BMD analyses. Graphs of the selected modeling results for BMDLs selected for use in risk estimation are also included below, where applicable.

[5] BMDS package version and timestamps: U.S. Environmental Protection Agency. (2025). BMDS Online (25.1; pybmds 25.1; bmdscore 25.1) [Software]. Available from https://bmdsonline.epa.gov, accessed January 26, 2026.

A.1 Cho et al. (2023)

A.1.1 Eosinophilic Globules (Nasal Cavity) in Male Mice

Dichotomous data for increased incidence of eosinophilic globules in the nasal cavity in male B6C3F1 mice exposed to *o*-dichlorobenzene via inhalation for 13 weeks (6 hours/day, 5 days/week) were modeled using a BMR of 10% ER consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). To dichotomize the response data, severity scores of 2 to 4 were counted as positive and scores of 0 to 1 were counted as negative. The sum of all animals in each dichotomous category (positive or negative) was used as model input.

For the HEC calculations, exposure concentrations were adjusted for duration from 6 hours/day to a full day (24 hours); concentrations were not further duration-adjusted from 5 days/week to 7 days/week because this dataset is being considered for an acute-duration point of departure (POD). Exposure concentrations were further adjusted for regional gas dose ratio (RGDR). The BMD modeling results for increased incidence of eosinophilic globules in male mice are summarized in Table_Apx A-1.

Table_Apx A-1. Summary of BMD Modeling Results for Eosinophilic Globules (Nasal Cavity) in Male Mice Exposed to *o*-Dichlorobenzene via Inhalation (Cho et al., 2023) [a]

Model	BMDL 10% ER (ppm)	BMD 10% ER (ppm)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Multistage 1 degree[c,d]	**0.289**	**0.427**	**36.26**	**-3.90E-4**	**S**	The Multistage 1-, 2-, and 3-degree, Gamma, Weibull, Quantal Linear, and Hill models provided adequate fit to the data (chi-square p-value > 0.1) and were considered viable. The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. The Multistage 1-, 2- and 3-degree, Gamma, Weibull, and Quantal Linear models all converged and these models had the lowest AIC.
Multistage 2 degree[c,d]	0.289	0.427	36.26	-3.90E-4	V	
Multistage 3 degree[c,d]	0.289	0.427	36.26	-3.90E-4	V	
Gamma[c,d]	0.289	0.427	36.26	-3.90E-4	V	
Weibull[c,d]	0.289	0.427	36.26	-3.90E-4	V	
Quantal Linear[c,d]	0.289	0.427	36.26	-3.90E-4	V	
Hill[d,e]	0.204	1.819	38.23	-2.22E-7	V	
Log Logistic	Lowest dose/BMDL ratio > 10.0				Q	
Log Probit	Lowest dose/BMDL ratio > 10.0 BMD/BMDL ratio > 20.0					
Logistic, Probit	Goodness-of-fit p-value < 0.1					

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model. Green shading indicates viable models. Gray shading indicates questionable models.
[c] Lowest dose/BMD ratio > 3.0.
[d] Lowest dose/BMDL ratio > 3.0.
[e] BMD/BMDL ratio > 3.0.

A.2 Hayes et al. (1985)

A.2.1 Maternal Absolute Body Weight in Rats on Gestation Day (GD) 16

Continuous data for decreased maternal absolute body weight on GD 16 in Fischer 344 rats exposed to *o*-dichlorobenzene via inhalation on GD 6 to GD 15 (6 hours/day) were modeled using BMRs of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for decreased maternal absolute body weight in rats on GD 16 are summarized in Table_Apx A-2.

Table_Apx A-2. Summary of BMD Modeling Results for Maternal Absolute Body Weight on GD 16 in Rats Exposed to *o*-Dichlorobenzene via Inhalation (Hayes et al., 1985) Using the Constant Variance Model[a]

Model	BMDL (ppm)	BMD (ppm)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model provided adequate fit to the variance data (Test 2 p-value > 0.05). Using a BMR of 1 SD, the Exponential 3, Exponential 5, Hill, Power, and Linear models provided adequate fit to the means (Test 4 p-value > 0.1). Using a BMR of 10% RD, the Exponential 3, Hill, Power, and Linear models provided adequate fit to the means (Test 4 p-value > 0.1). For both BMRs, the BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. The results for the BMR of 10% RD were dismissed because the BMDL for the recommended model was greater than the highest dose tested; therefore, the BMR of 1 SD was utilized.
Exponential 3[c]	126	161	810.91	0.32	S	
Hill[c]	139	259	812.05	-0.07	V	
Power[c]	125	158	811.01	0.33	V	
Linear[c]	125	158	811.01	0.33	V	
Polynomial 2, Polynomial 3	Goodness-of-fit p-value < 0.1				Q	
Exponential 5	Model did not successfully execute				U	
BMR: 1 SD						
Exponential 3	**49.0**	**64.3**	**810.91**	**-0.30**	**S**	
Exponential 5	27.6	50.3	812.08	0.27	V	
Hill	26.2	50.1	812.05	0.26	V	
Power	50.3	65.3	811.01	-0.34	V	
Linear	50.3	65.3	811.01	-0.34	V	
Polynomial 3	Goodness-of-fit p-value < 0.1				Q	
Polynomial 2	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.
[c] BMD/highest dose ratio > 1.0; BMDL/highest dose ratio > 1.0.

A.2.2 Maternal Body Weight Gain in Rats During GD 6-20

Continuous data for decreased maternal body weight gain during GD 6 to GD 20 in Fischer 344 rats exposed to *o*-dichlorobenzene via inhalation on GD 6 to GD 15 (6 hours/day) were modeled using a BMR of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for decreased maternal body weight gain in rats during GD 6-20 are summarized in Table_Apx A-3. The model results provided adequate fit only for 10% RD, which is less relevant to maternal body weight gain as an adversity benchmark for weight gain is not established. The selected BMDL value was discounted.

Table_Apx A-3. Summary of BMD Modeling Results for Maternal Body Weight Gain During GD 6-20 in Rats Exposed to ortho-Dichlorobenzene via Inhalation (Hayes et al., 1985) Using the Constant Variance Model[a]

Model	BMDL (ppm)	BMD (ppm)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model provided adequate fit to the variance data (Test 2 p-value > 0.05). The Exponential 3 model using a BMR of 10% RD provided adequate fit to the means (Test 4 p-value > 0.1) and was the only viable model; therefore, this model was selected.
Exponential 3	**32.8**	**49.2**	**904.76**	**-0.18**	**S**	
Exponential 5	Lowest dose/BMDL ratio > 10.0				Q	
Hill	Lowest dose/BMDL ratio > 10.0 BMD/BMDL ratio > 20.0					
Polynomial 2, Polynomial 3, Power, Linear	Goodness-of-fit p-value < 0.1					
BMR: 1 SD						
Exponential 3, Polynomial 2, Polynomial 3, Power, Linear	Goodness-of-fit p-value < 0.1				Q	
Exponential 5	Model did not successfully execute				U	
Hill	BMDL does not exist					

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.

A.2.3 Maternal Body Weight Gain in Rats During GD 6-8

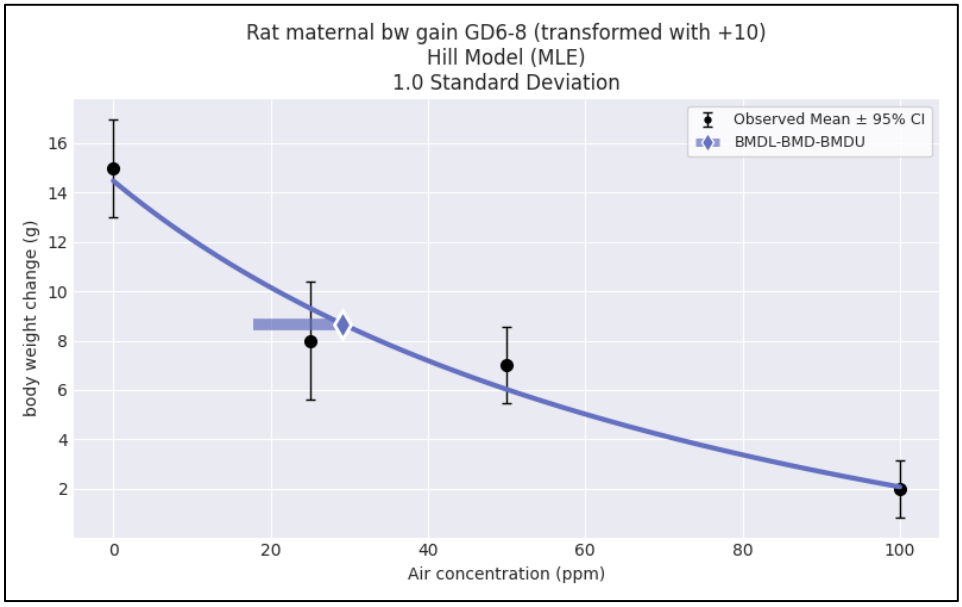
Continuous data for decreased maternal body weight gain during GD 6-8 in Fischer 344 rats exposed to *o*-dichlorobenzene via inhalation on GD 6-15 (6 hours/day) were modeled using a BMR of 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). A BMR of 10% RD was considered an inappropriate BMR because the transformed values for mean body weight gain are artificial for comparing absolute change, and the relevance of 10% RD to relative weight gain is unclear.

Because the weight gain was negative in exposed dams during GD 6-8, the data were transformed by adding 10 g to the mean weight gain value for each dose. The BMD modeling results for decreased maternal body weight gain in rats on GD 6-8 are summarized in Table_Apx A-4.

Table_Apx A-4. Summary of BMD Modeling Results for Maternal Body Weight Gain During GD 6-8 in Rats Exposed to *o*-Dichlorobenzene via Inhalation (Hayes et al., 1985) Using the Nonconstant Variance Model[a]

Model	BMDL 1 SD (ppm)	BMD 1 SD (ppm)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Hill	**17.8**	**29.2**	**647.91**	**-1.37**	**S**	The constant variance model did not provide an adequate fit to the variance data (Test 2 p-value < 0.05), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, only the Hill model provided adequate fit to the means (Test 4 p-value > 0.1); therefore, this model was selected.
Exponential 5	Zero degrees of freedom; saturated model				Q	
Exponential 3, Polynomial 2, Polynomial 3, Power, Linear	Goodness-of-fit p-value < 0.1					

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); Q = questionable model. Green shading indicates viable models. Gray shading indicates questionable models.



Figure_Apx A-1. BMD Modeling Graph of the Hill Model for Maternal Body Weight Gain During GD 6-8 in Rats

A.3 Umemura et al. (1996)

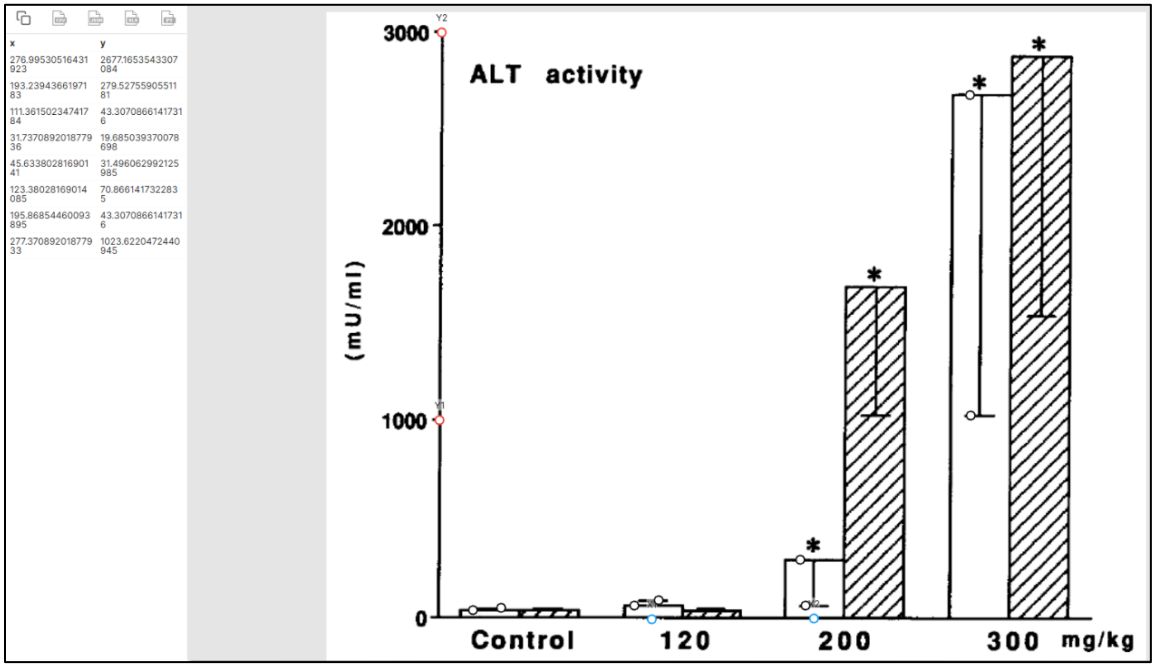
A.3.1 Serum ALT in Male Mice

Continuous data for increased serum ALT in male B6C3F1 mice following a single gavage administration of *o*-dichlorobenzene were modeled using a BMR of 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). Serum ALT values were obtained from bar graphs in (Umemura et al., 1996) using digitizing software (https://plotdigitizer.com/app, accessed January 26, 2026). The digitization results are shown in Figure_Apx A-2 and the BMD modeling results for increased serum ALT in male mice are summarized in Table_Apx A-5.

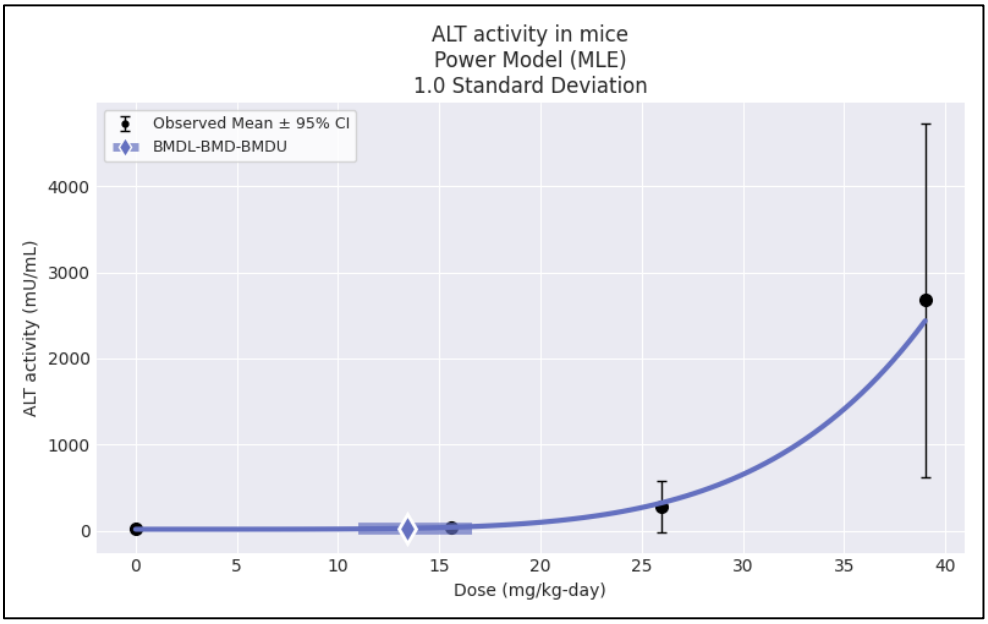
Table_Apx A-5. Summary of BMD Modeling Results for Serum ALT in Male Mice Exposed to *o*-Dichlorobenzene via a Single Gavage Dose (Umemura et al., 1996) Using the Nonconstant Variance Model[a]

Model	BMDL 1 SD (mg/kg)	BMD 1 SD (mg/kg)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Power	**11.0**	**13.4**	**248.98**	**0.07**	**S**	The constant variance model did not provide an adequate fit to the variance data (Test 2 p-value < 0.05), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, the Power and Exponential 3 models provided adequate fit to the means (Test 4 p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Exponential 3	5.68	8.62	252.04	-0.94	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	
Polynomial 3	Goodness-of-fit p-value < 0.1					
Polynomial 2, Linear	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.



Figure_Apx A-2. Plot Digitization of Serum ALT Results from (Umemura et al., 1996)



Figure_Apx A-3. BMD Modeling Graph of the Power Model for Serum ALT in Male Mice

A.4 Robinson et al. (1991)

A.4.1 Absolute Liver Weight in Male Rats

Continuous data for increased absolute liver weight in male SD rats administered *o*-dichlorobenzene via gavage for 90 days were modeled using BMRs of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased absolute liver weight in male rats are summarized in Table_Apx A-6.

Table_Apx A-6. Summary of BMD Modeling Results for Absolute Liver Weight in Male Rats Exposed to *o*-Dichlorobenzene via Gavage (Robinson et al., 1991) Using the Constant Variance Model[a]

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model provided adequate fit to the variance data (Test 2 p-value > 0.05). For both BMRs, the Power, Polynomial 2, Polynomial 3, Linear, and Exponential 3 models provided adequate fit to the means (Test 4 p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. The Power, Polynomial 2, Polynomial 3, and Linear models all converged (for each BMR) and these models had the lowest AIC.
Power	**27**	**38**	**171.20**	**1.29**	**S**	
Polynomial 2	27	38	171.20	1.29	V	
Polynomial 3	27	38	171.20	1.29	V	
Linear	27	38	171.20	1.29	V	
Exponential 3	31	42	171.51	1.35	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	
BMR: 1 SD						
Power	37	51	171.20	1.29	S	
Polynomial 2	37	51	171.20	1.29	V	
Polynomial 3	37	51	171.20	1.29	V	
Linear	37	51	171.20	1.29	V	
Exponential 3	41	55	171.51	1.35	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model. Green shading indicates viable models. Gray shading indicates questionable models.

A.4.2 Relative Liver Weight in Male Rats

Continuous data for increased relative liver weight in male SD rats administered *o*-dichlorobenzene via gavage for 90 days were modeled using a BMR of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased relative liver weight in male rats are summarized in Table_Apx A-7.

Table_Apx A-7. Summary of BMD Modeling Results for Relative Liver Weight in Male Rats Exposed to *o*-Dichlorobenzene via Gavage (Robinson et al., 1991) Using the Constant Variance Model[a]

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model provided adequate fit to the variance data (Test 2 p-value > 0.05). For both BMRs, all models provided adequate fit to the means (Test 4 p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. The Power, Polynomial 2, Polynomial 3, and Linear models all converged (for each BMR) and these models had the lowest AIC.
Power	**19**	**21**	**-7.03**	**0.46**	**S**	
Polynomial 2	19	21	-7.03	0.46	V	
Polynomial 3	19	21	-7.03	0.46	V	
Linear	19	21	-7.03	0.46	V	
Exponential 3	22	25	-6.11	0.81	V	
Exponential 5	10	16	-5.74	-0.24	V	
Hill	9.7	16	-5.75	-0.25	V	
BMR: 1 SD						
Power	14	18	-7.03	0.46	S	
Polynomial 2	14	18	-7.03	0.46	V	
Polynomial 3	14	18	-7.03	0.46	V	
Linear	14	18	-7.03	0.46	V	
Exponential 3	17	21	-6.11	0.81	V	
Exponential 5	8.4	13	-5.74	0.70	V	
Hill	8.0	13	-5.75	0.70	V	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model. Green shading indicates viable models.

A.4.3 Absolute Liver Weight in Female Rats

Continuous data for increased absolute liver weight in female SD rats administered *o*-dichlorobenzene via gavage for 90 days were modeled using BMRs of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased absolute liver weight in female rats are summarized in Table_Apx A-8.

Table_Apx A-8. Summary of BMD Modeling Results for Absolute Liver Weight in Female Rats Exposed to *o*-Dichlorobenzene via Gavage (Robinson et al., 1991) Using the Constant Variance Model[a]

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model provided adequate fit to the variance data (Test 2 p-value > 0.05). For both BMRs, the Linear, Polynomial 2, Polynomial 3, Power, and Exponential 3 models provided adequate fit to the means (Test 4 p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Linear[c]	**14**	**16**	**84.05**	**0.41**	**S**	
Polynomial 2[c]	14	16	84.05	0.41	V	
Power[c]	14	16	84.05	0.41	V	
Polynomial 3[c]	14	16	84.07	0.41	V	
Exponential 3[c]	18	20	85.20	0.89	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	
BMR: 1 SD						
Linear[c]	12	14	84.05	-0.08	S	

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Polynomial 2[c]	12	14	84.05	-0.08	V	The Linear, Polynomial 2, and Power models all converged (for each BMR) and these models had the lowest AIC.
Power[c]	12	14	84.05	-0.08	V	
Polynomial 3[c]	12	15	84.07	-0.12	V	
Exponential 3[c]	15	18	85.20	0.89	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model. Green shading indicates viable models. Gray shading indicates questionable models.
[c] Control SD fit > 1.5.

A.4.4 Relative Liver Weight in Female Rats

Continuous data for increased relative liver weight in female SD rats administered *o*-dichlorobenzene via gavage for 90 days were modeled using a BMR of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased relative liver weight in female rats are summarized in Table_Apx A-9.

Table_Apx A-9. Summary of BMD Modeling Results for Relative Liver Weight in Female Rats Exposed to *o*-Dichlorobenzene via Gavage (Robinson et al., 1991) Using the Nonconstant Variance Model[a]

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model did not provide an adequate fit to the variance data (Test 2 p-value < 0.05), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, the Linear and Power models provided adequate fit to the means (Test 4 p-value > 0.1) for both BMRs. The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Linear	**12**	**14**	**2.64**	**-1.13**	**S**	
Power	13	19	3.00	0.41	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	
Polynomial 2, Polynomial 3	Goodness-of-fit p-value < 0.1					
Exponential 3	Model did not successfully execute				U	
BMR: 1 SD						
Linear	7.6	9.8	2.64	-1.13	S	
Power	8.4	14	3.00	-1.02	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	
Polynomial 2, Polynomial 3	Goodness-of-fit p-value < 0.1					
Exponential 3	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.

A.4.5 Serum ALT in Male Rats

Continuous data for increased serum ALT in male SD rats administered *o*-dichlorobenzene via gavage for 90 days were modeled using a BMR of 1 SD consistent with EPA's *Benchmark Dose Technical Guidance.* The BMD modeling results for increased serum ALT in male rats are summarized in Table_Apx A-10.

Table_Apx A-10. Summary of BMD Modeling Results for Serum ALT in Male Rats Exposed to *o*-Dichlorobenzene via Gavage (Robinson et al., 1991) Using the Nonconstant Variance Model[a]

Model	BMDL 1 SD (mg/kg-day)	BMD 1 SD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	The constant variance model did not provide an adequate fit to the variance data (Test 2 p-value < 0.05), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, none of the models were considered viable; therefore, no model was selected.
Polynomial 2, Polynomial 3, Power, Linear	Goodness-of-fit p-value < 0.1					
Exponential 3	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: Q = questionable model; U = unusable model. Gray shading indicates questionable or unusable models.

A.4.6 Serum ALT in Female Rats

Continuous data for increased serum ALT in female SD rats administered *ortho*-dichlorobenzene via gavage for 90 days were modeled using a BMR of 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased serum ALT in female rats are summarized in Table_Apx A-11.

Table_Apx A-11. Summary of BMD Modeling Results for Serum ALT in Female Rats Exposed to *o*-Dichlorobenzene via Gavage (Robinson et al., 1991) Using the Nonconstant Variance Model[a]

Model	BMDL 1 SD (mg/kg-day)	BMD 1 SD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Linear	**19**	**35**	**294.98**	**0.19**	**S**	The constant variance model did not provide an adequate fit to the variance data (Test 2 p-value < 0.05), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, the Linear, Polynomial 2, Polynomial 3, and Power models provided adequate fit to the means (Test 4 p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Polynomial 2	20	43	296.68	0.46	V	
Polynomial 3	20	43	296.68	0.46	V	
Power	20	42	296.53	0.46	V	
Exponential 5, Hill	Zero degrees of freedom; saturated model				Q	
Exponential 3	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.

A.5 (NTP, 1985)

A.5.1 Relative Liver Weight in Male Rats

Continuous data for increased relative liver weight in male F344/N rats administered *o*-dichlorobenzene via gavage for 13 weeks (5 days/week) were modeled using BMRs of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased relative liver weight in male rats are summarized in Table_Apx A-12.

Table_Apx A-12. Summary of BMD Modeling Results for Relative Liver Weight in Male Rats Exposed to *o*-Dichlorobenzene via Gavage (NTP, 1985) Using the Nonconstant Variance Model[a]

Model	BMDL 10% RD and 1 SD (mg/kg-day)	BMD 10% RD and 1 SD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Exponential 3, Exponential 5, Hill, Polynomial 2, Polynomial 3, Power, Linear	Goodness-of-fit p-value < 0.1				Q	The constant variance model did not provide an adequate fit to the variance data (Test 2 p-value < 0.05), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, none of the models provided adequate fit to the means (Test 4 p-value > 0.1); therefore, no model was selected.

[a] Data are shown only for models that were considered viable.
[b] Model fit: Q = questionable model. Gray shading indicates questionable models.

A.5.2 Relative Liver Weight in Female Rats

Continuous data for increased relative liver weight in female F344/N rats administered *o*-dichlorobenzene via gavage for 13 weeks (5 days/week) were modeled using a BMR of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased relative liver weight in female rats are summarized in Table_Apx A-13.

Table_Apx A-13. Summary of BMD Modeling Results for Relative Liver Weight in Female Rats Exposed to *o*-Dichlorobenzene via Gavage (NTP, 1985) Using the Constant Variance Model[a]

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR 10% RD						The constant variance model provided an adequate fit to the variance data (Test 2 p-value > 0.05). For both BMRs, all models provided adequate fit to the means (Test 4 p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Linear	**23.93**	**27.80**	**-20.49**	**0.34**	**S**	
Exponential 3	26.80	30.37	-20.44	0.50	V	
Exponential 5	19.84	30.38	-16.69	0.55	V	
Hill	19.72	30.41	-16.70	0.55	V	
Polynomial 2	23.93	28.84	-18.52	0.40	V	
Polynomial 3	23.93	28.71	-18.52	0.40	V	
Power	23.96	29.57	-18.56	0.45	V	
BMR: 1 SD						
Linear	15.29	18.54	-20.49	0.34	S	
Exponential 3	17.23	20.53	-20.44	0.50	V	
Exponential 5	12.93	21.58	-16.69	0.55	V	
Hill	12.82	21.65	-16.70	0.55	V	

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Polynomial 2	15.31	19.32	-18.52	0.40	V	
Polynomial 3	15.32	19.23	-18.52	0.40	V	
Power	15.33	20.06	-18.56	0.45	V	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model. Green shading indicates viable models.

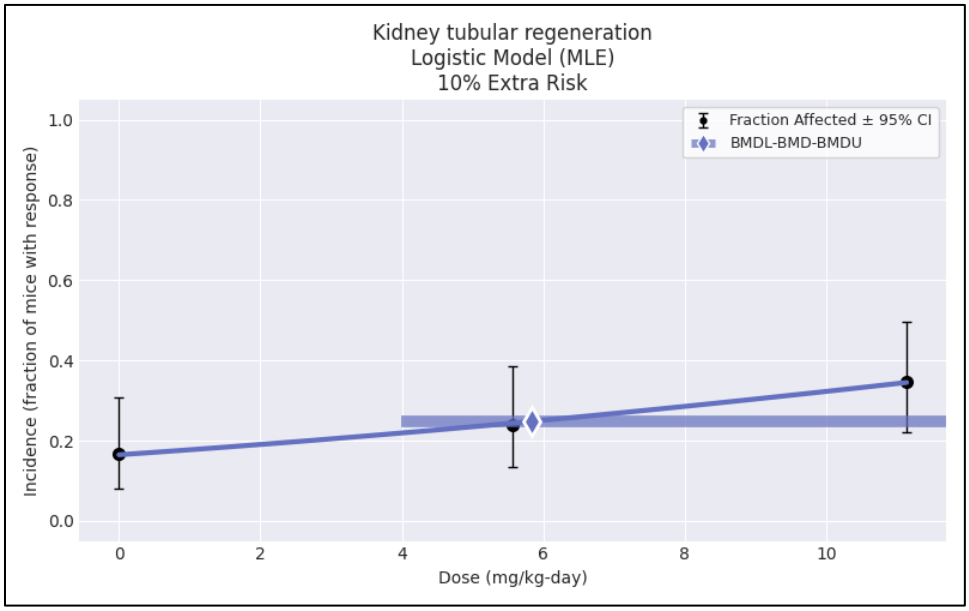
A.5.3 Kidney Tubule Regeneration in Male Mice

Dichotomous data for increased incidence of kidney tubule regeneration in male B6C3F1 mice exposed to *o*-dichlorobenzene via gavage for 2 years (5 days/week) were modeled using a BMR of 10% ER consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased incidence of kidney tubule regeneration in male mice are summarized in Table_Apx A-14.

Table_Apx A-14. Summary of BMD Modeling Results for Kidney Tubular Regeneration in Male Mice Exposed to *o*-Dichlorobenzene via Gavage (NTP, 1985)

Model	BMDL 10% ER (mg/m^3)	BMD 10% ER (mg/m^3)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Logistic	**3.996**	**5.845**	**165.63**	**-0.062**	**S**	The Logistic, Multistage 1, Probit, and Quantal Linear models provided adequate fit to the data (chi-square p-value > 0.1). The BMDLs of the viable models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Multistage 1	2.746	5.004	165.71	-0.237	V	
Probit	3.825	5.718	165.64	-0.086	V	
Quantal Linear	2.746	5.004	165.71	-0.237	V	
Gamma, Log Logistic, Multistage 2, Weibull	Zero degrees of freedom; saturated model				Q	
Hill, Log Probit	Zero degrees of freedom; saturated model; BMDL does not exist				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable and unusable models.



Figure_Apx A-4. BMD Modeling Graph of Logistic Model for Kidney Tubular Regeneration in Male Mice

A.6 NIEHS (2025a)

A.6.1 Serum ALT in Female Mice

Continuous data for increased serum ALT in female B6D2F1/Crl mice following 5 days of *o*-dichlorobenzene exposure via inhalation for 6 h/day were modeled using a BMR of 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The dataset used for model input is provided in (U.S. EPA, 2026b). The BMD modeling results for increased serum ALT in female mice are summarized in Table_Apx A-15. The dose-response of the dataset was impacted by the 1 ppm dose group, which showed a decrease in serum ALT compared to controls, primarily due to an outlier animal sample. All unrestricted models were attempted.

Table_Apx A-15. Summary of BMD Modeling Results for Serum ALT in Female Mice Exposed to *o*-Dichlorobenzene via Inhalation (NIEHS, 2025a)[a]

Model	BMDL 1 SD (mg/kg)	BMD 1 SD (mg/kg)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Exponential 3, Exponential 5, Hill, Polynomial 2, Polynomial 3, Power, Linear	Control stdev. fit > 1.5; Goodness of fit p-value < 0.1; Constant variance test failed (Test 2 p-value < 0.05)				Q	The constant, non-constant, and linear variance models did not provide an adequate fit to the variance data (Test 2 p-value < 0.05). This held true even with all unrestricted models used. EPA also attempted to model the dataset with the 1 ppm dose group removed, or with the outlier from the 1 ppm dose group removed, however the models produced the same conclusions in both cases.
Polynomial 3 (unrestricted)	Same as above plus BMD/BMDL ratio > 3.0				Q	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.

A.6.2 Absolute Liver Weight in Female Mice

Continuous data for increased absolute liver weight in female B6D2F1/Crl mice administered following 5 days of *o-dichlorobenzene* exposure via inhalation for 6 h/day were modeled using a BMR of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD modeling results for increased absolute liver weight in female mice are summarized in Table_Apx A-16. Default unrestricted models were run.

Table_Apx A-16. Summary of BMD Modeling Results for Absolute Liver Weight in Female Mice Exposed to *o*-Dichlorobenzene via Inhalation (NIEHS, 2025a) Using the Nonconstant Variance Model[a]

Model	BMDL (mg/kg-day)	BMD (mg/kg-day)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
BMR: 10% RD						The constant variance model did not provide an adequate fit to the variance data for all models (Goodness of fit p-value < 0.1; residual near BMD > 2.0), but the nonconstant variance model provided adequate fit (Test 3 p-value > 0.05). With the nonconstant variance model applied, the Hill model provided adequate fit to the means (Test 4 p-value > 0.1) for both BMRs.
Hill	**22**	**23**	**-119.28**	**0.005**	**S**	
Exponential 5, Hill, Polynomial 2, Polynomial 3, Power. Linear	Goodness of fit p-value < 0.1; residual near BMD > 2.0				Q	
Exponential 3	Did not successfully execute				U	
BMR: 1 SD						
Hill	10.1	22.1	-119.28	0.005	V	
Exponential 5	Goodness-of-fit p-value < 0.1				Q	
Exponential 5, Hill, Polynomial 2, Polynomial 3, Power. Linear	Goodness of fit p-value < 0.1; residual near BMD > 2.0				Q	
Exponential 3	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.

A.6.3 Relative Liver Weight in Female Mice

Continuous data for increased relative liver weight in female B6D2F1/Crl mice administered following 5 days of *o-dichlorobenzene* exposure via inhalation for 6 h/day were modeled using a BMR of 10% RD and 1 SD consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). The BMD

modeling results for increased relative liver weight in female mice are summarized in Table_Apx A-17. Default unrestricted models were run.

Table_Apx A-17. Summary of BMD Modeling Results for Relative Liver Weight in Female Mice Exposed to *o*-Dichlorobenzene via Inhalation (NIEHS, 2025a)[a]

Model	BMDL 1 SD (mg/kg)	BMD 1 SD (mg/kg)	AIC	Scaled Residual Near BMD	Fit[b]	Model Selection Notes
Exponential 5, Hill, Polynomial 3, Power	Control stdev. fit > 1.5; Goodness of fit p-value < 0.1; Constant variance test failed (Test 2 p-value < 0.05)				Q	The constant, non-constant, and linear variance model all did not provide an adequate fit to the variance data. Only the default unrestricted models were run because absolute liver weight was successfully modeled using that model set.
Polynomial 2	Goodness of fit p-value < 0.1; Residual near BMD >2				Q	
Linear	Residual at control > 2.0; Goodness of fit p-value < 0.1; Residual near BMD >2				Q	
Exponential 3	Model did not successfully execute				U	

[a] Data are shown only for models that were considered viable.
[b] Model fit: S = selected viable model (bold indicates the model being considered for POD derivation); V = viable model; Q = questionable model; U = unusable model. Green shading indicates viable models. Gray shading indicates questionable or unusable models.

Appendix B OTHER HUMAN HEALTH HAZARD OUTCOMES

This appendix discusses organ systems that have weaker existing evidence integration conclusions, insufficient information, and /or reported effects at exposures similar to or higher than the critical human health hazard outcomes identified in Section 2.3.1, *e.g.,* respiratory toxicity, liver toxicity, kidney toxicity, and changes in body weight). Taken together, none of the following outcomes were considered for dose-response and POD derivation. See the full data extraction for all relevant studies in *Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology* (U.S. EPA, 2026d).

As described in Section 2.1.1, EPA reviewed prior assessments from ATSDR and EPA IRIS in 2006 (ATSDR, 2006; U.S. EPA, 2006c) and used the database summaries from those assessments to help focus this analysis. The IRIS assessment was a draft that was never finalized. As such, this *Other Human Health Hazard Outcomes* narrative summarizes information from the ATSDR report unless otherwise stated. As analyzed and reported by ATSDR in 2006, information on outcomes in humans is limited to observations in the respiratory tract and eye irritation in workers following chronic exposure to *o*-dichlorobenzene vapor. New studies for these other outcomes since the 2006 ATSDR assessment were limited or did not add to the hazard database (https://hawc.epa.gov/summary/visual/assessment/100500091/o-dichlorobenzene-human-health-and-environmental-h/, accessed March 10, 2026). EPA determined further analysis was not needed for these outcomes.

B.1 Reproductive and Developmental Toxicity

Human Evidence

EPA did not identify any reasonably available information assessing reproductive and developmental effects of *o*-dichlorobenzene exposure in humans.

Laboratory Animal Evidence

In a two-generation inhalation study on rats (Biodynamics, 1989), there were no reported adverse effects on reproductive performance, fertility, or offspring in either generation. There was also an absence of reproductive and developmental outcomes following gestational exposure of pregnant rats and rabbits (Hayes et al., 1985). In that study, the only reported adverse outcome was reduced maternal body weight, which was discussed among the broader hazard database for body weight changes (Section 2.3.1.1.2).

Repeated dose systemic toxicity studies did not report any effects on male or female reproductive organs. In an older study testing different lengths of exposure on male rats and guinea pigs, there were no reported changes in testicular weight or histology via inhalation exposed to 93 ppm *o*-dichlorobenzene for 6-7 months (Hollingsworth et al., 1958). Similarly, there was no histopathology of male or female reproductive organs or changes in organ weights in rats following 10 or 90 days of oral administration to as high as 400 mg/kg-day (Robinson et al., 1991). The same was true for both mice and rats following 13 weeks of up to 500 mg/kg-day or 2 years of up to 120 mg/kg-day (NTP, 1985).

Mechanistic and Supporting Evidence

There is no evidence for disruption of reproductive hormones or signaling following *o*-dichlorobenzene exposure (Appendix B.3).

Evidence Integration Summary

Based on a complete absence of any adverse effect on reproductive organs or outcomes across various study designs covering multiple species, there is strong evidence that *o*-dichlorobenzene exposure does not result in reproductive or developmental toxicity.

B.2 Hematologic and Immune System Toxicity

Human Evidence

As summarized in (ATSDR, 2006), infrequent industrial hygiene surveys and medical examinations, sampling and methods unspecified, were conducted in a plant where an unreported number of men were exposed to *o*-dichlorobenzene at an average level of 15 ppm (range 1–44 ppm) for an unreported duration (Hollingsworth et al., 1958). No effects on clinical hematology indices (red blood cell (RBC) count, total and differential white blood cell (WBC) counts, hemoglobin, hematocrit, and mean corpuscular volume) were reported to be "…attributable to exposure to *o*-dichlorobenzene...."

Laboratory Animal Evidence

As summarized in (ATSDR, 2006), there was some varying evidence of hematologic and immune system toxicity following both vapor inhalation and oral exposures to *o*-dichlorobenzene.

One study in 5 male SD rats exposed to 0, 5, 10, 16, or 29 ppm *o*-dichlorobenzene for 4 hours found total WBC counts were significantly ($p \leq 0.05$) reduced, also known as leucopenia, at ≥10 ppm without any changes in WBC differential or RBC counts (Brondeau et al., 1990). This same study further assessed the effect of *o*-dichlorobenzene on total WBC count in groups of 10 male SD rats that were normal or adrenalectomized and exposed to 0 or 24 ppm for 4 hours. Adrenalectomy caused a significant increase in total WBCs (39.9% higher than normal controls), although exposure did not significantly affect WBC count in the adrenalectomized rats.

Unlike the other irritants tested in Brondeau et al. (1990), the study authors concluded *o*-dichlorobenzene-induced leucopenia may be independent of irritation stress, having occurred at lower vapor concentrations not previously reported to cause irritation. This was not noted in the ATSDR analysis for *o*-dichlorobenzene, which dismissed the effect. Thus, the mechanism proposed for the other irritants tested, that adrenal-dependent leucopenia was a secondary manifestation of increased secretion of glucocorticosteroids and thus associated with sensory irritation, was not concluded to be immediately applicable to *o*-DCB (Brondeau et al., 1990).

No hematological changes were reported in rabbits (2/sex) or monkeys (2 females) exposed to 93 ppm *o*-dichlorobenzene for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al., 1958). The specific hematology end points were not specified.

Absolute spleen weight or spleen histology for rats (at least 17/sex) or guinea pigs (at least 7/sex) exposed to 93 ppm *o*-dichlorobenzene for 7 hours/day, 5 days/week for 6–7 months has been

evaluated (ATSDR, 2006; Hollingsworth et al., 1958). The study reported that rats had no appreciable change in absolute spleen weight. Histological evaluations were not specified but declared as normal in the male guinea pigs despite reported significant decreases in absolute spleen weight.

In the Robinson et al. study, groups of 10 male and 10 female SD rats were treated with *o*-dichlorobenzene in corn oil by gavage at doses of 0, 37.5, 75, 150, or 300 mg/kg-day for 10 consecutive days (Robinson et al., 1991). Dose selection was based on a reported rat oral LD50 of 500 mg/kg. Statistically significant changes in organ weights reportedly predominantly occurred at 300 mg/kg-day, including significantly decreased absolute spleen (both sexes) and thymus (males) weights. The NTP 1985 13 week toxicology study in 10/sex Fischer 344/N rats, and 10/sex B6C3F1 mice, administered *o*-dichlorobenzene in corn oil by gavage for doses 0, 30, 60, 125, 250, and 500 mg/kg, reported minimal hematological changes (NTP, 1985). These changes occurred at the highest doses tested, 500 mg/kg, included slight decreases in rat hemoglobin and hematocrit (both sexes), slightly decreased RBC counts (significant in male rats), and lymphoid depletion in the spleen and thymus (both sexes) (NTP, 1985). Significantly increased leukocyte counts for male rats in the 150 and 300 mg/kg-day dose groups were also concluded to be *o*-dichlorobenzene treatment-related compared to controls (Robinson et al., 1991). Hollingsworth et al. (1958) also reported a slight decrease in absolute spleen weight of female "white" rats exposed to 18.8, 188, and 376 mg/kg *o*-dichlorobenzene in olive oil by stomach tube, 5 days/week, over 192 days (Hollingsworth et al., 1958). There were no corresponding changes reported in hematological endpoints that were not specified (Hollingsworth et al., 1958).

ATSDR previously summarized no changes in absolute spleen weight or spleen histology for rats (20/sex) or guinea pigs (8/sex) exposed to 93 ppm *o*-dichlorobenzene for 7 hours/day, 5 days/week for 6–7 months (ATSDR, 2006; Hollingsworth et al., 1958). Histological evaluations were not specified but declared as normal.

More recent studies identified since the 2006 ATSDR assessment have focused on *o*-dichlorobenzene as a component of environmentally persistent free radicals (EPFRs) because of combustion-generated particulate matter. Given the focus of these studies on *o*-dichlorobenzene as part of a mixture sorbed to silica and copper (II) oxide, these studies were not considered further for hazard (Mahne et al., 2012; Lord et al., 2011).

Mechanistic and Supporting Evidence

The hematologic, immune, and inflammation effects of *o*-dichlorobenzene can be complex given its irritant properties (Robinson et al., 1991).

Erythropoietin promotes the production of red blood cells (Thomas et al., 2005) and is synthesized in renal tubules, which could be affected by male rat-specific α2μ-globulin kidney toxicity. The NTP study found a significant decrease in RBC counts in male rats (NTP, 1985).

Evidence Integration Summary

While there is evidence for blood or immune system toxicity in animals at elevated doses and potentially associated with α2μ-globulin kidney toxicity that is not relevant to humans, there is

limited observations in humans. These hematologic and immune effects are not more sensitive than the human health hazard endpoints identified in Section 2.6, and thus the proposed PODs would be protective.

B.3 Endocrine Organ Toxicity

Human Evidence

Consistent with ATSDR's 2006 assessment (ATSDR, 2006), EPA did not identify any reasonably available information assessing endocrine effects in humans, specifically for adrenal and thyroid glands, following *o*-dichlorobenzene exposure.

Laboratory Animal Evidence

Limited studies evaluated endocrine endpoints including gross examinations, organ weights (Robinson et al., 1991), and microscopic examinations (NTP, 1985) following *o*-dichlorobenzene exposure. No adverse effects, including for adrenal, thyroid, and pituitary glands, were reported for doses up to 500 mg/kg-day *o*-dichlorobenzene.

Mechanistic and Supporting Evidence

EPA did not identify any relevant information assessing endocrine effects of *o*-dichlorobenzene exposure.

Evidence Integration Summary

There is indeterminate evidence to evaluate endocrine organ toxicity from *o*-dichlorobenzene exposure.

B.4 Cardiovascular Toxicity

Human Evidence

EPA did not identify any reasonably available information assessing cardiovascular effects of *o*-dichlorobenzene exposure in humans.

Laboratory Animal Evidence

As summarized by ATSDR (ATSDR, 2006), no changes in absolute heart weight or heart histology were reported for rats (20/sex), guinea pigs (8/sex), rabbits (2/sex), or monkeys (2 females) following exposure to 93 ppm *o*-dichlorobenzene for 7 hours/day, 5 days/week for 6–7 months, or in mice (10 females) that were similarly exposed to 49 ppm *o*-dichlorobenzene (Hollingsworth et al., 1958). Summarized studies did not specify histopathological examinations.

Multifocal mineralization of the myocardial fibers of the heart was found in B6C3F1 mice (3/10 males, 8/10 females) that were administered 500 mg/kg-day of *o*-dichlorobenzene in corn oil by gavage 5 days/week for 13 weeks (NTP, 1985); this effect does not appear to have occurred in controls or lower dose mice (≤250 mg/kg-day).

More recent studies identified since the 2006 ATSDR assessment have focused on *o*-dichlorobenzene as a component of EPFRs because of combustion-generated particulate matter. Despite exposure to particulate matter has known adverse health impacts, *o*-dichlorobenzene as part of a mixture sorbed to silica and copper (II) oxide did not enable these mixture studies to be considered further for hazard (Mahne et al., 2012; Lord et al., 2011).

Mechanistic and Supporting Evidence

EPA did not identify any relevant information assessing cardiovascular toxicity effects of *o*-dichlorobenzene exposure.

Evidence Integration Summary

There is indeterminate evidence to evaluate cardiovascular toxicity from *o*-dichlorobenzene exposure.

B.5 Ocular Toxicity

Human Evidence

As summarized by ATSDR (ATSDR, 2006), infrequent industrial hygiene surveys and medical examinations were conducted in a plant where an unreported number of men were exposed to *o*-dichlorobenzene at an average level of 15 ppm (range 1–44 ppm) for an unreported duration (Hollingsworth et al., 1958). No eye or nasal irritation was reportedly attributable to exposure. Researchers performing the Hollingsworth et al. (1958) repeated vapor inhalation experiments on animals also sensed the odor of *o*-dichlorobenzene at a concentration of 50 ppm without eye or nasal irritation. An earlier source (Elkins 1950) referenced by Hollingsworth et al. (1958) reported that occupational exposure to 100 ppm of *o*-dichlorobenzene caused irritation of the eyes and respiratory passages.

More recent studies identified since the 2006 ATSDR assessment have focused on *o*-dichlorobenzene as a component of EPFRs because of combustion-generated particulate matter. One study using the case-control investigation of air pollution exposure among California Children, the Air Pollution and Childhood Cancer study, examined the relationship between "ambient exposure to air toxics" and retinoblastoma (Heck et al., 2015), including *o*-dichlorobenzene. Correlation to *o*-dichlorobenzene was not concluded. This study was discussed in context of cancer hazard (Section 2.4.2.1).

Laboratory Animal Evidence

Limited animal studies, including in rats (n = 5–20), rabbits (n = 2), reported observations of eye irritation following vapor exposure to *o*-DCB (Hollingsworth et al., 1958). However, most of these studies had limited to no methods nor complete reporting of results.

More recent studies identified since the 2006 ATSDR assessment have focused on *o*-dichlorobenzene as a component of EPFRs because of combustion-generated particulate matter. Given the focus of these studies on *o*-dichlorobenzene as part of a mixture sorbed to silica and copper (II) oxide, these studies were not considered further for hazard (Mahne et al., 2012; Lord et al., 2011).

Mechanistic and Supporting Evidence

o-dichlorobenzene is not considered acidic or basic, so the mechanism for eye irritation is unknown. The above studies are based on vapor exposure, however exposure to *o*-dichlorobenzene powder would likely cause mechanical irritation.

Evidence Integration Summary

There is slight evidence for eye irritation and indeterminate evidence for any other ocular toxicity from exposure to *o*-dichlorobenzene vapor. These ocular effects are not more sensitive than the human health hazard endpoints identified in Section 2.6, and thus the proposed PODs would be protective against ocular toxicity. At least one study concluded that potentially toxic effects can occur prior to irritation (Brondeau et al., 1990) lending to intermediate evidence that ocular irritation and toxicity is not the most sensitive endpoint for *o*-dichlorobenzene.

B.6 Musculoskeletal Toxicity

Human Evidence

EPA did not identify any reasonably available information assessing musculoskeletal effects in humans following *o*-dichlorobenzene exposure in humans.

Laboratory Animal Evidence

As summarized by ATSDR (ATSDR, 2006), multifocal mineralization of skeletal muscle was found in B6C3F1 mice (3/10 males, 8/10 females) that were administered 500 mg/kg-day of *o*-dichlorobenzene in corn oil by gavage 5 days/week for 13 weeks (NTP, 1985); this effect does not appear to have occurred in controls or lower dose mice (≤250 mg/kg-day). No gross or histological changes were observed in muscle of B6C3F1 mice treated with ≤120 mg/kg-day, 5 days/week for 103 weeks (NTP, 1985), or in SD or F344 rats that treated with 300 mg/kg-day for 10 days (Robinson et al., 1991), ≤500 mg/kg-day, 5 days/week for 13 weeks (NTP, 1985), or ≤120 mg/kg-day, 5 days/week for 103 weeks (NTP, 1985).

ATSDR as previously summarized that no gross or histological changes in bone were observed in any of the rat or mouse 10-day, 13-week, or 103-week studies (Robinson et al., 1991; NTP, 1985).

Mechanistic and Supporting Evidence

EPA did not identify any relevant information assessing musculoskeletal toxicity effects of *o*-dichlorobenzene exposure.

Evidence Integration Summary

There is indeterminate evidence to evaluate musculoskeletal toxicity from *o*-dichlorobenzene exposure. Further, the musculoskeletal effects observed in the NTP 1985 study are not more sensitive than the human health hazard endpoints identified in Section 2.6, and thus the proposed PODs would be protective against musculoskeletal toxicity.

B.7 Gastrointestinal Toxicity

Human Evidence

EPA did not identify any reasonably available information assessing gastrointestinal effects in humans following *o*-dichlorobenzene exposure in humans.

Laboratory Animal Evidence

As summarized by ATSDR (ATSDR, 2006), the limited studies that performed gross and histological examinations of the gastrointestinal tract found no changes in rats or mice up to 500 mg/kg-day exposure to *o*-dichlorobenzene (Robinson et al., 1991; NTP, 1985).

Mechanistic and Supporting Evidence

EPA did not identify any relevant information assessing musculoskeletal toxicity effects of *o*-dichlorobenzene exposure.

Evidence Integration Summary

There is indeterminate evidence to evaluate gastrointestinal toxicity from *o*-dichlorobenzene exposure.

Appendix C 5-DAY WHOLE-BODY INHALATION STUDY MECHANISTIC GENE EXPRESSION ANALYSIS AND TRANSCRIPTOMIC PODS (tPOD) NOT CONSIDERED FOR USE IN RISK ESTIMATION

C.1 Mechanistic Gene Expression Analysis

EPA acquired the raw sequencing data (FASTQ) files generated by NIEHS for the 5-day *in vivo* whole-body inhalation transcriptomic study (NIEHS, 2025a) to perform exploratory qualitative and quantitative analyses. EPA analyzed gene expression by performing dose-response modeling on each individual gene of the 5-day transcriptomic data for mice and rats (U.S. EPA, 2026l). Individual gene responses in lung, heart, liver, kidney, and ovary were subsequently grouped into predefined collections, or gene sets, to interpret and provide more biological context to the observed transcriptional changes using publicly available gene sets including the Molecular Signatures Database (MSigDB) (Castanza et al., 2023; Liberzon et al., 2015; Subramanian et al., 2005). The MSigDB has coverage over an extremely diverse landscape of biological mechanisms with 50,236 total unique accession numbers for gene sets in the version used for this analysis. This broad representation of potential toxicities creates a valuable background for untargeted examination of potential chemical-induced transcriptomic effects. MSigDB gene sets with at least three genes were categorized into the following groups of expertly curated terms called "supersets": apoptosis, cytotoxicity, inflammation, oxidative stress, necrosis, or a combination of all five terms. MSigDB was queried for these superset gene sets using the following string-matching terms: "apoptosis", "cytotox", "inflam", "oxidative", or "necrosis". These cellular processes were consistent with tissue and cell toxicity observed in toxicity studies following *o*-dichlorobenzene exposure. Further support for these categories of genes were evident in the apical effects observed following exposure to *o*-dichlorobenzene, reflecting the specific mechanistic key events (KEs) previously described including general toxicity and cell death (U.S. EPA, 2026l; Mörbt et al., 2011; ATSDR, 2006; U.S. EPA, 2006c; Zissu, 1995; Hayes et al., 1985; NTP, 1985; Haskell, 1982; Hollingsworth et al., 1958). The results of these concentration-responsive gene expression changes are provided below for three out of the five organs examined in the NIEHS 5-day whole-body inhalation study following *o*-dichlorobenzene exposure in female rodents.

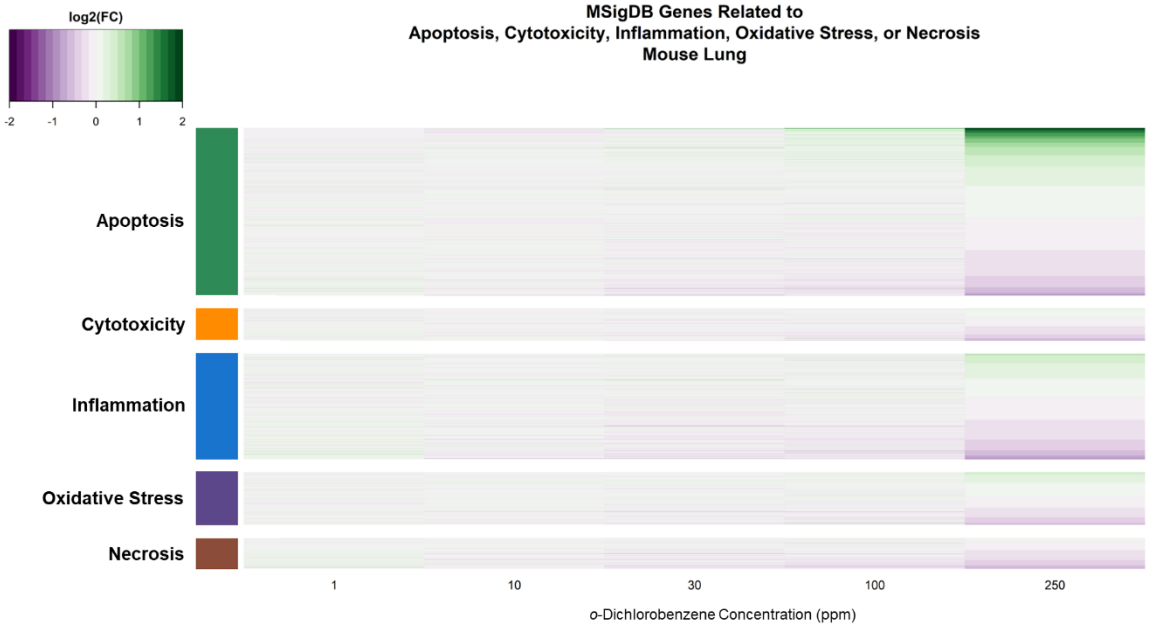
Differential expression of the MSigDB supersets occurred primarily at the highest concentrations tested (250 ppm in mice, 500 ppm in rats), but they provide evidence that *o*-dichlorobenzene exposure results in transcriptomic changes within biologically relevant gene sets of interest.

More results of these transcriptomic analyses and related methods are provided in EPA's technical report, *Supporting Hazard Characterization of 1,2-Dichlorobenzene and 1,4-Dichlorobenzene Using an EPA 5-Day in Vivo Transcriptomic Study Protocol* (U.S. EPA, 2026l).

C.1.1 Lung

Mechanistic gene expression results for the lung were visualized as heatmaps across the full landscape of genes within MSigDB supersets.

Differential expression of the MSigDB supersets occurred primarily at the highest concentrations tested (250 ppm in mice), but they provide evidence that *o*-dichlorobenzene exposure results in transcriptomic changes within biologically relevant gene sets of interest in the lung. Overall, the mouse lung shows a greater relative *increase* in gene expression for these cell damage-related supersets at the highest exposure concentration of 250 ppm.



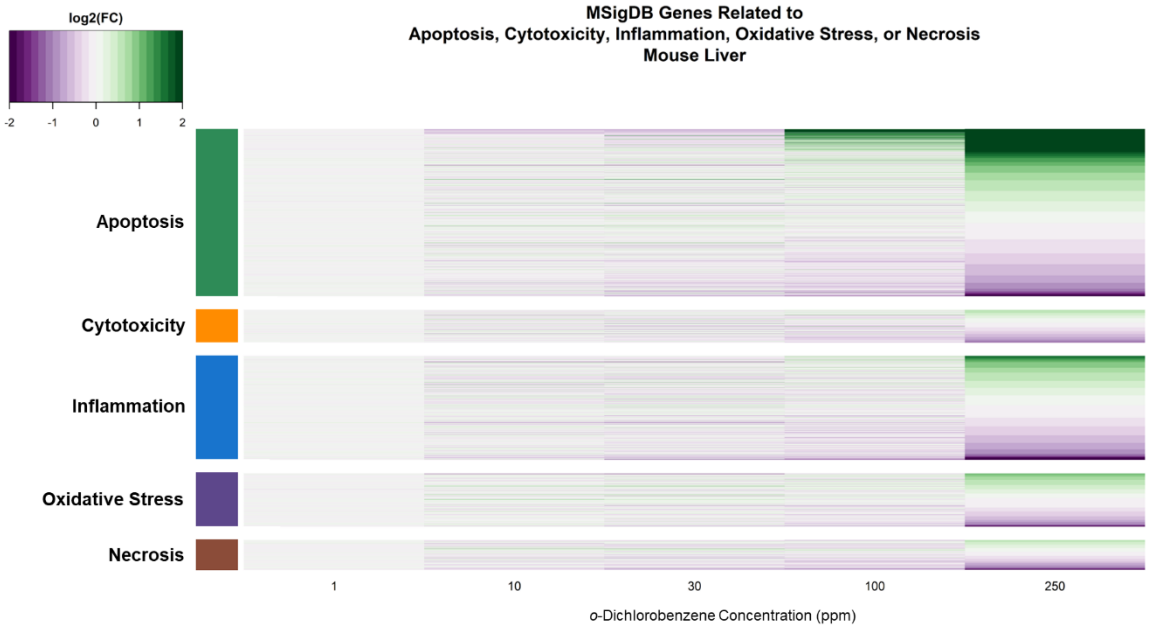
Figure_Apx C-1. Heatmap of the Mouse Lung Gene Set Deregulation (represented as log_2 fold change) Following *o*-Dichlorobenzene Exposure of each Gene Annotated to the Five MSigDB Supersets

Upregulated genes are displayed in green and downregulated genes in purple with color density indicating the relative strength of the change.

C.1.2 Liver

Mechanistic gene expression results for liver were visualized as heatmaps across the full landscape of genes within MSigDB supersets (Figure_Apx C-2 for mouse).

In mice, there was robust upregulation of genes at 250 ppm for multiple superset terms, with a lesser but still visually evident upregulation of genes at 100 ppm. These data provide evidence that *o*-dichlorobenzene exposure results in transcriptomic changes within each gene set of interest in the liver that may be relevant to the observed apical outcomes.



Figure_Apx C-2. Heatmap of the Mouse Liver Gene Set Deregulation (represented as log_2 fold change) Following *o*-Dichlorobenzene Exposure of each Gene Annotated to the Five MSigDB Supersets

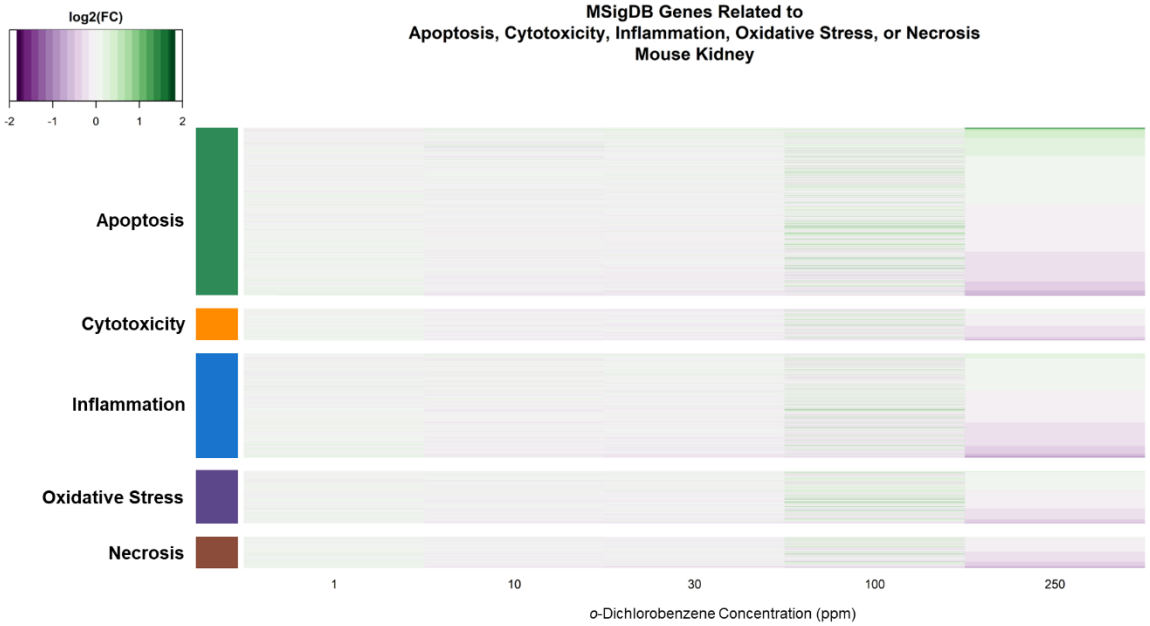
Upregulated genes are displayed in green and downregulated genes in purple with color density indicating the relative strength of the change.

C.1.3 Kidney

Mechanistic gene expression results for kidney were visualized as heatmaps across the full landscape of genes within MSigDB supersets.

In mice, there was minimal transcriptomic response even at the highest dose, with only mild activation or suppression of gene expression across the five supersets. The mouse results indicated a subtle dysregulation of gene expression at 100 ppm, with more pronounced

disruption at the highest concentration of 250 ppm; however, the specific genes changes were largely not dose-responsive.



Figure_Apx C-3. Heatmap of the Mouse Kidney Gene Set Deregulation (represented as log_2 fold change) Following *o*-Dichlorobenzene Exposure of each Gene Annotated to the Five MSigDB Supersets

Upregulated genes are displayed in green and downregulated genes in purple with color density indicating the relative strength of the change.

C.2 Transcriptional PODs Based on ETAP Framework

In the ETAP approach (U.S. EPA, 2024a), the tPOD represents the median BMD lower bound (BMDL) value of the genes within the Gene Ontology Biological Process (GOBP) class with the single lowest median BMD value meeting all inclusion criteria. The GO framework categorizes biological knowledge into three domains, with Biological Process describing the events within a biological activity (*e.g.,* immune response), Molecular Function describing specific biochemical activities of a gene product (*e.g.,* kinase activity), and Cellular Component describing the location where the gene product functions (*e.g.,* nucleus). While the ETAP method of identifying tPODs gives an indication of overall sensitivity across species and organs, the coordinated transcriptional changes used to identify the POD do not necessarily discriminate between specific hazards, adverse or adaptive effects, nor are they intended to infer a mechanism or MOA.

In addition to the three target organs of liver, lung, and kidney, ovary and kidney were also analyzed. The $GOBP_{low}$ tPOD value for *o*-dichlorobenzene was identified as 0.73 ppm in mouse (ovary). Among the three target organs, the most sensitive tPOD for mouse was 1.01 ppm in kidney. Table_Apx C-1 lists the tPODs across all tested tissues following exposure to *o*-dichlorobenzene.

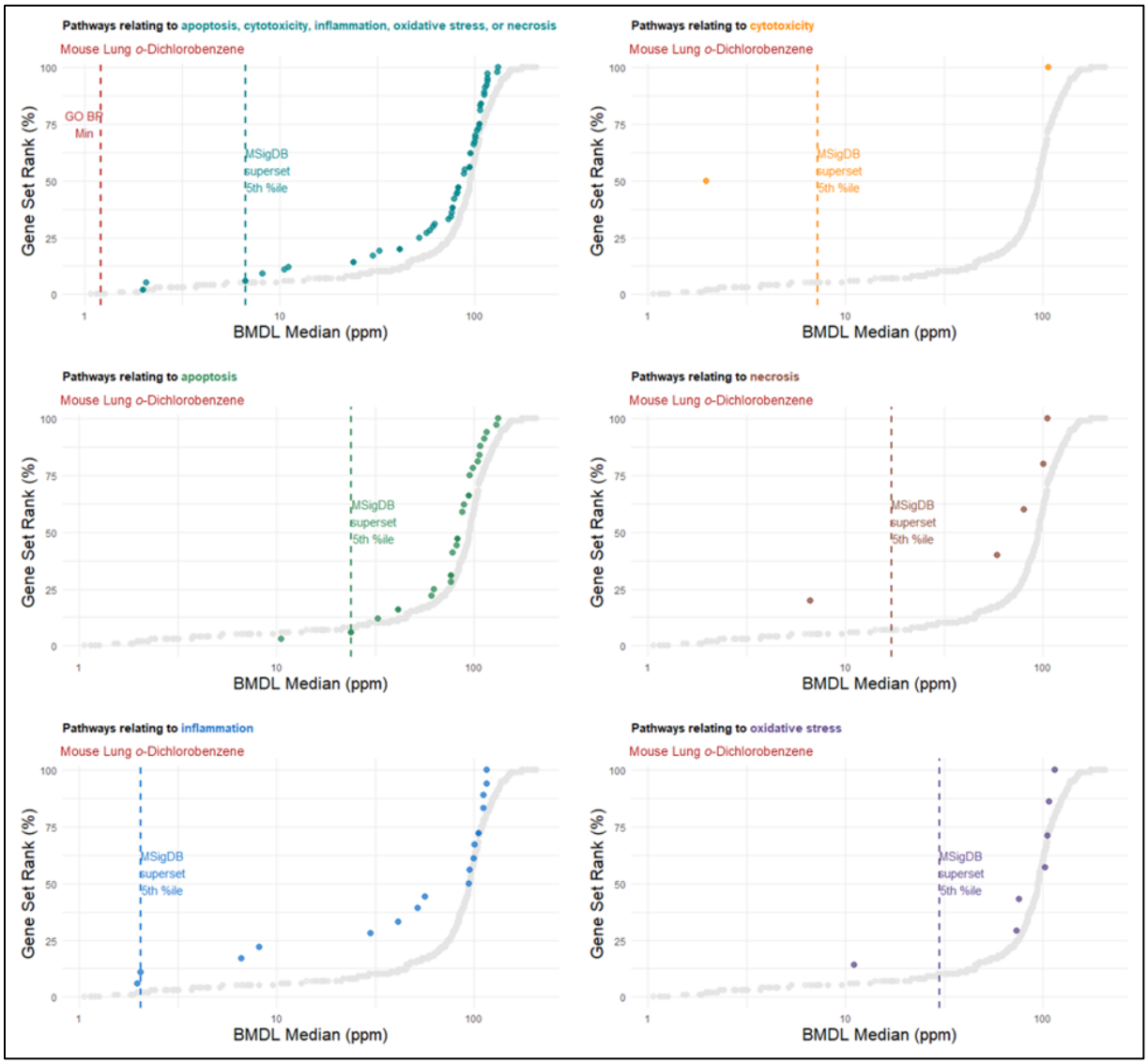
Table_Apx C-1. Lowest GO Biological Process Class Median BMD Values across Tissues in Female Rats and Mice Treated with *o*-Dichlorobenzene

Tissue	Species	GO Accession	GO Biological Process Class	# of Genes with BMD	Median BMD (ppm)	Median BMDL (ppm)
Heart	Mouse	GO:0006730	one-carbon metabolic process	3	4.03	1.09
Kidney	Mouse	GO:0007623	circadian rhythm	3	4.15	1.01
Liver	Mouse	GO:0071397	cellular response to cholesterol	3	4.65	1.22
Lung	Mouse	GO:0001843	neural tube closure	3	4.35	1.21
Ovary	**Mouse**	**GO:0045933**	**positive regulation of muscle contraction**	**3**	**3.07**	**0.73[a]**

[a] The median BMD lower bound value associated with the GO biological process class with the lowest median benchmark dose value across all tissues in bold was selected as the tPOD.

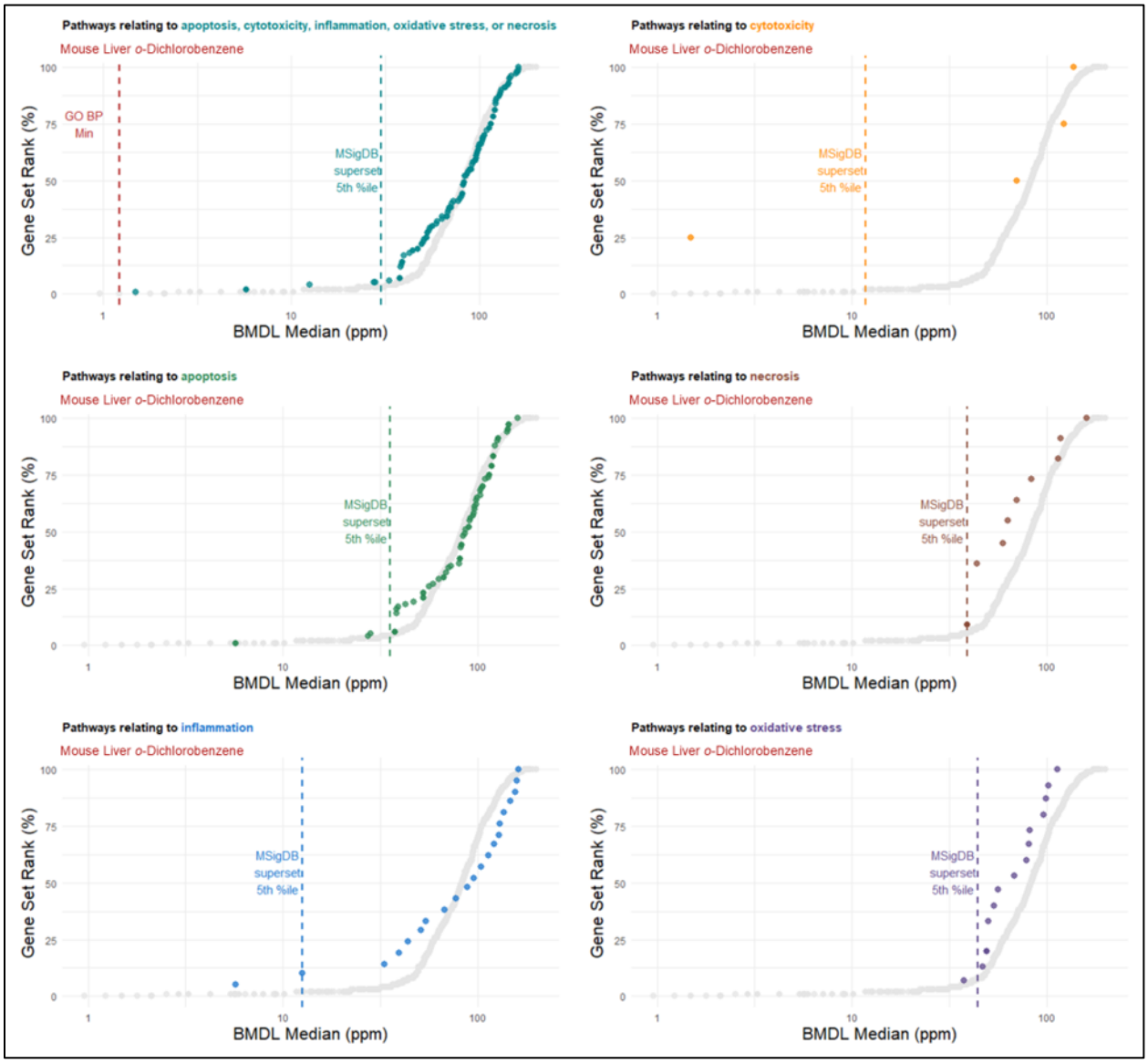
C.3 Accumulation Plots for Superset-Specific tPODs

C.3.1 Lung



Figure_Apx C-4. Mouse Lung Accumulation Plots Showing the Gene Set Ranks (%)
All gene sets in the MSigDB database are shown with grayed circles; each circle represents a distinct MSigDB gene set term. The combined gene set with any term from apoptosis, cytotoxicity, inflammation, necrosis, and oxidative stress, and including at least three genes with BMD meeting all inclusion criteria, is shown against the landscape of all gene sets in color, with each subsequent plot highlighting a specific subset of those gene sets. The vertical dashed red line highlights the $GOBP_{low}$ tPOD, the others highlight either the MSigDB $SUPER_{0.05}$ tPOD or the specific 5th percentile tPOD for the five individual supersets.

C.3.2 Liver



Figure_Apx C-5. Mouse Liver Accumulation Plots Showing the Gene Set Ranks (%)
All gene sets in the MSigDB database are shown with grayed circles; each circle represents a distinct MSigDB gene set term. The combined gene set with any term from apoptosis, cytotoxicity, inflammation, necrosis, and oxidative stress, and including at least three genes with BMD meeting all inclusion criteria, is shown against the landscape of all gene sets in color, with each subsequent plot highlighting a specific subset of those gene sets. The vertical dashed red line highlights the $GOBP_{low}$ tPOD, the others highlight either the MSigDB $SUPER_{0.05}$ tPOD or the specific 5th percentile tPOD for the five individual supersets.

C.4 Dose-Response Analysis for Transcriptional POD from Kidney Tissue

Mouse Results

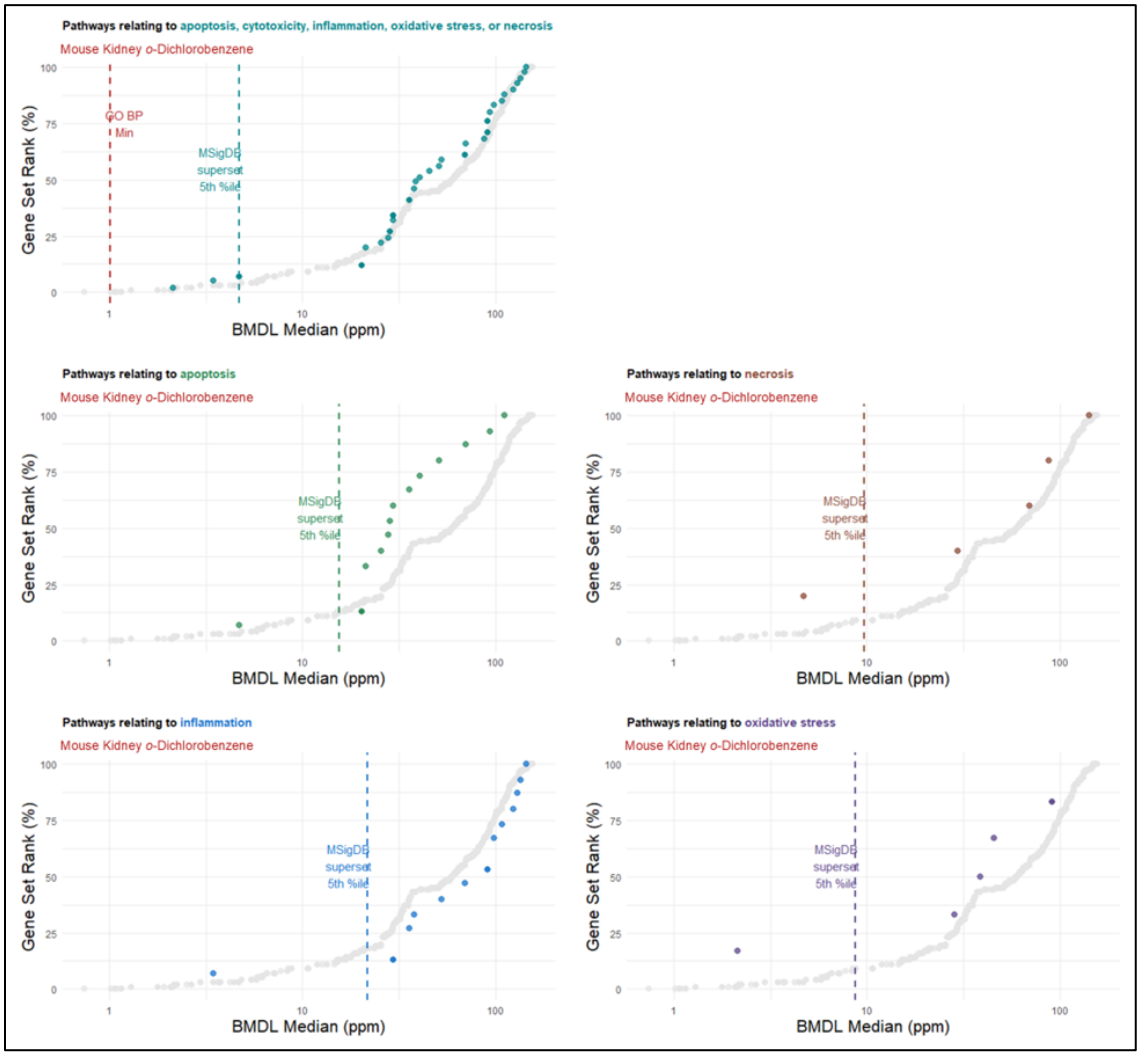
Constraining the BMD results to MSigDB gene sets involved in apoptosis, cytotoxicity, inflammation, oxidative stress, necrosis, or a combination of all five individual terms revealed that the most sensitive MSigDB superset 5th percentile BMDL median in the mouse kidney was for oxidative stress gene sets (most sensitive BMDL median = 2.12 ppm, 5th percentile BMDL median = 8.68 ppm). No cytotoxicity-related gene sets were induced for mouse kidney, so BMDLs could not be derived for that superset. The 5th percentile BMDL median across the MSigDB combined four supersets = 4.72 ppm. Results are summarized in Table_Apx C-2. A closer analysis of the tissue sampling also indicated that there were few mouse kidney samples available due to a contamination issue, with some samples showing increased expression of pancreas-specific genes (U.S. EPA, 2026l; NIEHS, 2025a). Therefore, mouse kidney results are uncertain overall.

Table_Apx C-2. BMD Modeling Results of Targeted Gene Sets Relating to Apical Effects from *o*-Dichlorobenzene Exposure (Apoptosis, Cytotoxicity, Inflammation, Oxidative Stress, Necrosis) within Mouse Kidney

	Gene Set Accession	Lowest BMDL Median (ppm)	5th Percentile BMDL Median (ppm)
GOBP	GO:0007623	1.01	6.20
MSigDB[a]	M2581	0.74	6.18
MSigDB Combined Superset[b]	MM15823	2.12 (oxidative stress)	4.72
MSigDB Oxidative Stress	MM15823	2.12	8.68
MSigDB Inflammation	MM9519	3.44	21.71
MSigDB Apoptosis	MM11663	4.72	15.56
MSigDB Necrosis	MM6985	4.72	9.68

Note that there were no cytotoxicity-related gene sets identified in mouse kidney.
The lowest BMDL median for GOBP represents the BMDL of the lowest GO term rank ordered by BMD Median. The 5th percentile BMDL Median for GOBP represents the 5th percentile of GO terms rank ordered by BMDL Median.
The MSigDB and MSigDB superset results presented here are rank ordered by gene set-level BMDL Median and report the lowest and distribution-based 5th percentile gene set BMDL Medians for comparison.
For gene set accessions, the "GO" prefix refers to "Gene Ontology" accession identifiers, while the "MM" prefix refers to "mouse MSigDB" accession identifiers.
[a] The full MSigDB collection of gene sets.
[b] The Combined MSigDB Superset represents a consensus of multiple individual gene sets associated with a specific biological response. For this evaluation, cytotoxicity, apoptosis, inflammation, oxidative stress, and necrosis were prioritized.

Because the 5th percentile gene set BMDL median was below, but within approximately an order of magnitude of, the inflection point of the BMDL median accumulation plot curves, EPA determined that this dose most closely approximated the BMDL median just prior to maximal pathway activation and thus was able to capture more sensitive (yet not overly sensitive) gene set expression changes. EPA therefore only considered the 5th percentile BMDL medians for comparison to apical hazard values. Figure_Apx C-6 presents accumulation plots of the gene set ranks associated with each MSigDB superset as well as the combined superset in mouse kidney overlaid with the full landscape of MSigDB gene sets and compared to the $GOBP_{low}$ tPOD.



Figure_Apx C-6. Mouse Kidney Accumulation Plots showing the Gene Set Ranks (%) All gene sets in the MSigDB database are shown with grayed circles; each circle represents a distinct MSigDB gene set term. The combined gene set with any term from apoptosis, cytotoxicity, inflammation, necrosis, and oxidative stress, and including at least three genes with BMD meeting all inclusion criteria, is shown against the landscape of all gene sets in color, with each subsequent plot highlighting a specific subset of those gene sets. The vertical dashed red line highlights the $GOBP_{low}$ tPOD, the others highlight either the MSigDB $SUPER_{low}$ tPOD or the specific lower BMDL Median tPOD for the four individual supersets. Note that there were no cytotoxicity-related gene sets identified in mouse kidney.

C.4.1 Comparison of Transcriptomic PODs to Apical PODs

For kidney, the corresponding apical PODs were from mice as well. Therefore, despite being supplementary, these tPODs can be compared to their corresponding apical POD values.

tPOD HEC Derivation

The transcriptomic study (U.S. EPA, 2026l; NIEHS, 2025a) exposed mice and rats to *o*-dichlorobenzene for 6 hours a day for 5 consecutive days. Consistent with HEC adjustments made to apical PODs, tPOD HECs are adjusted to continuous exposed (24 hours/day). No dosimetric adjustment is required for systemic effects (*i.e.,* kidney) because relative blood:air partition coefficient defaults to 1.0.

The apical kidney POD was from an oral study, so the tPOD must be converted to oral doses for comparison using the equations in Section 2.3. In addition to using the 5th percentile endpoint-specific tPOD, for the purposes of comparison EPA used the $MSigDB_{0.05}$, defined as the 5th percentile of the distribution of gene set-level BMDLs combined across all five supersets, since the specific MOA for the apical effects are unknown. Despite the tPOD coming from a 5-day study, the derived HED is three-fold less than the apical HED from a chronic study. While this does demonstrate that superset tPODs can serve as a sensitive early marker even for chronic toxicity, this result is highly uncertain given the small sample size in the gene set, the questionable relevance to chronic adverse responses, and limited support for any mechanism of toxicity.

Table_Apx C-3. Comparison of Adjusted Superset-Specific tPODs with Apical PODs for Liver and Kidney

Organ	Study tPOD	tPOD HED	Apical HED	tPOD more sensitive?
Kidney	4.72 ppm	1.30 mg/kg-day	4.0 mg/kg-day	Yes

C.4.1.1 Conclusions

This analysis represents a novel approach to deriving PODs from transcriptomic data to support risk assessment and promote the use of the best available science. While the liver tPOD is directly relatable to the apical POD, there are significant uncertainties for the other tissues with corresponding adverse health outcomes.

The kidney tPOD is based on the same tissue in the same species, however there is insufficient mechanistic information underlying the cause of the kidney damage (observed as tubular regeneration in (NTP, 1985)). There is also additional statistical uncertainty in the mouse tPOD results due to a small sample size of both tissue samples and activated genes. Additionally, kidney toxicity appears to require sustained, long-term exposure for the majority of a rodent's lifetime, so there is substantial uncertainty in interpreting the transcriptomic response from a 5-day exposure. While these concerns would preclude using the tPOD for risk estimation, the tPOD-HED is approximately three-fold lower than the apical POD, indicating that it represents a sensitive early marker of potential toxicity.

The lung tPOD is in the same species as the apical POD for respiratory toxicity, but a different tissue (nasal/olfactory tissue) that demonstrates substantially higher toxicity. On the other hand,

there is some mechanistic support for a role of apoptosis and oxidative stress in lung tissue toxicity (Mörbt et al., 2011). Despite the diminished sensitivity of lung compared to nasal tissue, the tPOD is still slightly lower than the adjusted equivalent NOAEC for nasal tissue damage. This suggests that if a tPOD were available for nasal tissue, it could be several fold more sensitive and might represent a more precise molecular indicator of toxicity compared to the LOAECs from existing toxicity studies.

Appendix D HUMAN HEALTH HAZARD CONFIDENCE SUMMARY

Table_Apx D-1 summarizes the confidence ratings for each factor for the critical human health hazard endpoints and associated hazard values considered for acute, intermediate, and chronic non-cancer scenarios, and cancer lifetime exposure scenarios. The bolded rows in the table are the most robust and sensitive health effect for each exposure scenario and will be used to calculate risks for *o*-dichlorobenzene. "Slight" is indicated by a single +, moderate by ++, and robust by +++.

Table_Apx D-1. Confidence Summary for Human Health Hazard Assessment

Hazard Domain	Evidence Integration Conclusion	Selection of Most Critical Endpoint and Study	Relevance to Exposure Scenarios	Dose-Response Considerations	PESS Sensitivity	Overall Hazard Confidence
Acute/Intermediate/Chronic non-cancer						
Respiratory	+ +	+ + +	+ +	++	+ +	**Robust**
Liver	+ + +	+ + +	+ + +	+ ++	+ +	**Robust**
Lifetime cancer						
Cancer	Quantitative cancer risk assessment is not needed					Moderate-to-Robust

+ + + Robust confidence suggests thorough understanding of the scientific evidence and uncertainties. The supporting weight of scientific evidence outweighs the uncertainties to the point where it is unlikely that the uncertainties could have a significant effect on the hazard estimate.
+ + Moderate confidence suggests some understanding of scientific evidence and uncertainties. The supporting scientific evidence weighed against the uncertainties is reasonably adequate to characterize hazard estimates.
+ Slight confidence is assigned when the weight of scientific evidence may not be adequate to characterize the scenario, and when the assessor is making the best scientific assessment possible in the absence of complete information. There are additional uncertainties that may need to be considered.
Bolded rows indicate the endpoints used for risk estimation.

Appendix E IDENTIFICATION OF CANDIDATE ANALOGS FOR *o*-DICHLOROBENZENE

Candidate analogs for *o*-dichlorobenzene were collected from 19 resources, including two existing analog analyses conducted by the U.S. EPA, and additional searches using computational tools including the Organisation for Economic C*o*-operation and Development (OECD) Quantitative Structure-Activity (QSAR) Toolbox, U.S. EPA CompTox Chemicals Dashboard, Generalized Read-Across (GenRA), and Cheminformatics Modules. The details of each resource, including the settings and parameters used with computational tools, are provided in Table_Apx E-1. Each of the search strategies employed a unique methodology for identifying candidate analogs. Including multiple sources and search parameters ensures that different aspects of the target chemical's structure and properties are considered. This results in a more comprehensive and diverse pool of candidate analogs than would be generated using any single search by itself. The complete compilation of potential analogs and application of selection criteria can be found in *Draft Candidate Analog and Toxicity Values to Support ReCAAP Analysis for o-Dichlorobenzene* (U.S. EPA, 2026m).

Table_Apx E-1. Parameters of Tools and Resources Used for Read-Across Evaluation of *o*-Dichlorobenzene

Similarity Context [# of Candidate Analogs Identified][a]	Tool/Resource Name (Version)	Settings/Parameters [# Identified][b]	Searched by (Date)[c,d]
Structural [1,654][d]	Environmental Hazard Framework	• U.S. EPA-generated list developed following the Draft Framework for Selecting Analogs in Environmental Hazard Assessment [20]	CASRN/ Chemical Name (2024)

	U.S. EPA Initial Analysis	• U.S. EPA-generated Cheminformatics Modules:
		  ○ Substructure/Similarity > 0.85 [12]
		  ○ ToxPrints Similarity > 0.85 [11]
		• U.S. EPA-generated GenRA results:
		  ○ Morgan Fingerprints top 10 [10]
		  ○ ToxPrints top 10 [10]
		  ○ AIM 1st pass [7]
		  ○ PhysChem top 10 [10]
		  ○ Toxicity-ToxRef data top 10 [10]
			CASRN/ Chemical Name (Spring 2025)

	U.S. EPA CompTox Chemicals Dashboard (Version 2.5.3)	• Similar compounds - Tanimoto similarity threshold of 0.8 [51]	CASRN/ SMILES (July-August 2025)
		• Related substances [5]	
	U.S. EPA GenRA (GenRA 3.4, June 2025)	No filter (all data): • Morgan Fingerprints (top ~100 nearest neighbors) [84] • ToxPrints Fingerprints (top ~100 nearest neighbors) [46] • AIM Fingerprints (top ~100 nearest neighbors) [88] • PhysChem Fingerprints (top ~100 nearest neighbors) [99] • Torsion Fingerprints (top ~100 nearest neighbors) [61]	

Similarity Context [# of Candidate Analogs Identified][a]	Tool/Resource Name (Version)	Settings/Parameters [# Identified][b]	Searched by (Date)[c,d]
	U.S. EPA Cheminformatics Modules (DEV version, build: 2025-08-28)	• Similarity search with ≥80% similarity threshold: ○ AIM Chemotypes Version 1.0 - Tanimoto [584] ○ ToxPrints Chemotypes Version 2.0 - Tanimoto [90] • Substructure search: ○ Chlorobenzene structure, Use similarity, Bingo Fingerprints - Tanimoto [81]	
	OECD QSAR Toolbox (Version 4.7.1)	• Similarity search with ≥80% similarity threshold using default settings, all Human Health databases (46 selected), and all Inventories (11 selected); searches run with simplified user interface [1,015][d]: ○ Dice similarity ○ PubChem features ○ Hologram calculation ○ Combine all features ○ Atom characteristics: Atom type, Count H attached, and Hybridization	

[a] Number of unique candidate analogs identified using analog identification search tools.
[b] Count of unique candidate analogs identified by the tools; not included in the count are the target chemical, duplicate analogs based on CASRN provided by the tools, and analogs with no CASRN or invalid CASRN.
[c] SMILES collected from the U.S. EPA CompTox Chemicals Dashboard batch search of the structural analogs' CASRNs.
[d] *o*-Dichlorobenzene SMILES: ClC1=C(Cl)C=CC=C1 (CASRN: 95-50-1).

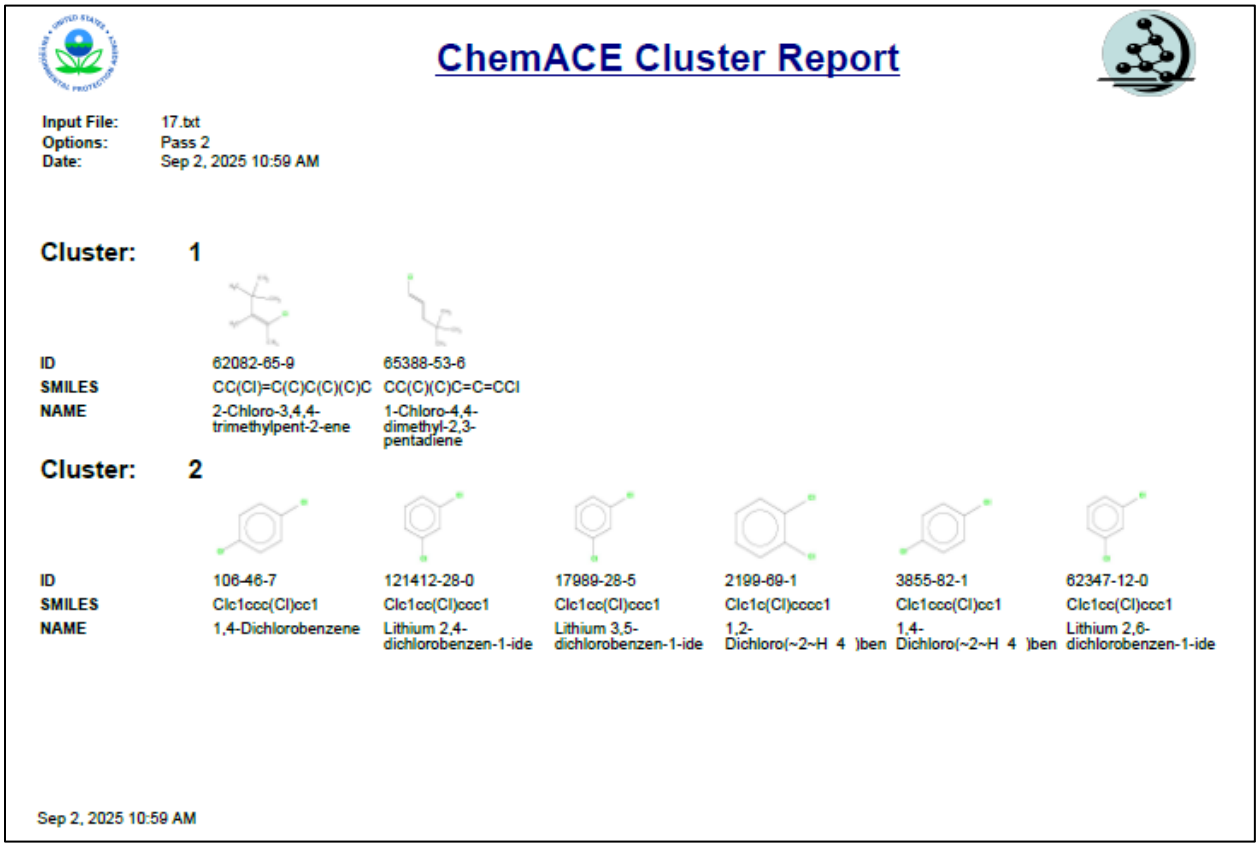
AIM = Analog Identification Methodology; CASRN = Chemical Abstracts Service Registry Number; GenRA = Generalized Read-Across; OECD = Organisation for Economic C*o*-operation and Development; QSAR = quantitative structure-activity relationship; SMILES = Simplified Molecular Input Line Entry System; U.S. EPA = U.S. Environmental Protection Agency.

The candidate analogs were collected, sorted, selected, and ranked for each tool output set according to the workflow outlined in Figure_Apx E-1.

Figure_Apx E-1. Workflow for Analog Identification, Selection, and Ranking

Each list of candidate analogs generated from the individual searches (resources, settings, and parameters for each search provided in Table_Apx E-1) was collected as a list of Chemical Abstracts Service Registry Numbers (CASRNs) for the candidate substances. Chemical names and QSAR-ready Simplified Molecular Input Line Entry System (SMILES) notations for each substance in the list were obtained from U.S. EPA's CompTox Chemicals Dashboard (https://www.epa.gov/comptox-tools/comptox-chemicals-dashboard, accessed November 5, 2025). QSAR-ready SMILES have salts, isotopes, and stere*o*-specific notations removed for

more consistent interpretation by models. The SMILES strings were used to generate chemical structures for screening and to prepare input files for the Chemical Assessment Clustering Engine (ChemACE). The candidates were sorted into structurally related groups or clusters using ChemACE to facilitate review. A sample ChemACE output is shown in Figure_Apx E-2. Candidate analogs without SMILES strings in the Dashboard, such as chemicals not included in the Dashboard, mixtures, and polymers, were separated into a batch for manual review.



Figure_Apx E-2. Sample ChemACE Output

E.1 Selection Criteria

The curated and sorted lists of candidate chemicals were reviewed based on selection criteria to identify potential analogs suitable for further review. Selection parameters were developed for screening the candidate analogs and ranking the selected potential analogs by preference. These criteria were based on structural elements that can affect overall structural similarity, including molecular size, shape, substituents, and flexibility, and on structural elements that affect reactivity, including the number and types of substituents allowed on the aromatic ring. These criteria prioritize potential analogs similar in reactivity and key physical-chemical properties (*e.g.,* water solubility and octanol/water partition coefficient [log K_{ow}]) to the target *o*-dichlorobenzene. In addition, inclusion parameters and preferences were refined based on metabolism information for *o*- and *p*-dichlorobenzene (ATSDR, 2006; U.S. EPA, 2006a). Both compounds exhibit similar metabolic pathways, with cytochrome P450 (CYP)-mediated ring hydroxylation predominating, forming phenols, catechols, quinones, and their conjugates. The metabolism data suggest that suitable analogs will have chemically and metabolically stable

aromatic-halogen bonds and that halogen displacement is not a likely mechanism contributing to toxicity.

Potential analogs for *o*-dichlorobenzene include halogenated benzenes containing chlorine, fluorine, and/or bromine. Bromine and fluorine are adjacent to chlorine in the periodic table and bromine shares a similar size and reactivity to chlorine when bound to aromatic rings. Although fluorine is smaller than chlorine and forms stronger bonds with lower reactivity, compounds containing fluorine may be suitable analogs because metabolism data for *o*-dichlorobenzene indicate that halogen displacement is unlikely. Iodobenzene compounds were excluded because iodine is significantly larger, forms weaker carbon-iodine bonds, and is more reactive relative to chlorine. Iodine-substituted benzenes may therefore introduce reactivity and mechanisms of toxicity not relevant to the target *o*-dichlorobenzene.

In a comprehensive approach, halogenated benzenes with single methyl groups on the ring were included for consideration, although they were lower in preference due to potential differences in metabolism from the possible oxidation of the methyl group. Halo-alkyl benzenes were excluded because their chemical reactivity differs from the target compound. Specifically, chloroalkyl benzenes contain a halogen on a benzylic position that is more susceptible to substitution, which could lead to differences in metabolic, degradation, and toxicity mechanisms compared to the target chemical.

Chemicals were excluded from further consideration if they contained isotopes or metals, had multiple rings (*e.g.,* biphenyls or other polycyclic structures), or contained non-target functional groups. Phenolic compounds that were identified as metabolites of *o*-dichlorobenzene, along with their simple (*e.g.,* sodium) salts, were included.

The criteria used to select potential analogs are summarized as follows:

- All elements other than carbon, hydrogen, chlorine, bromine, and fluorine should be excluded to preserve structural and functional similarity to the target chemical. Dichlorophenols may be included if they are potential metabolites of *o*-dichlorobenzene.
- Compounds should be halogenated benzenes containing chlorine, bromine, and/or fluorine, with chlorine and bromine preferred, due to their structural and functional similarity to the target.
- Halogenated benzenes with iodine should be excluded because the benzene-iodine bond can generate radicals, which may introduce differences in toxicity, and because iodine is significantly larger than the other halogens.
- The total number of halogens on the ring should be between one and four, with one to three preferred, to maintain similarity in structure, function, physical-chemical properties, and metabolic pathways. The total number of fluorine atoms should be no greater than two to avoid substances like per- and polyfluoroalkyl substances (PFAS).
- Compounds with multiple aromatic rings (such as biphenyl) and fused aromatic rings (such as naphthalene) should be excluded due to differences in molecular size, physical-chemical properties, and potential metabolites.
- A single methyl substituent on the ring may be included when combined with one to three halogens (chlorine preferred over bromine or fluorine), as this preserves structural

and functional similarity to the target. Compounds without methyl substituents are preferred because the methyl group may introduce metabolic pathways not relevant to the target chemical.

- Compounds with halogens on alkyl substituents should be excluded because they may act as alkylating agents, which may introduce differences in toxicity.
- Isotope-enriched compounds, such as deuterated or ^{13}C-labeled analogs, should be excluded because relevant data are unlikely to be available and their toxicokinetics may differ from non-isotope-enriched compounds.

Finally, the selected potential analogs for each set were ranked by preference. The order of preference, from highest to lowest, was: (1) chlorinated benzenes with one to three chlorines and potential metabolites of *o*-dichlorobenzene; (2) halogenated benzenes with one to three halogens (combinations of bromine, chlorine, and/or fluorine, with chlorine and bromine preferred); (3) tetra-chlorinated benzenes; (4) tetra-halogenated benzenes containing combinations of bromine, chlorine, and/or fluorine; (5) chlorinated methylbenzenes; and (6) halogenated methylbenzenes that include fluorine or bromine.

E.2 Unique Potential Analogs for *o*-Dichlorobenzene

The 19 search strategies described in Table_Apx E-1 resulted in a pool of 1,654 unique candidate analogs for *o*-dichlorobenzene. After applying the selection criteria, a combined total of 100 unique potential analogs were identified (Table_Apx E-2). The top 10 preferred potential analogs from each search listed in Table_Apx E-1 were combined and deduplicated, resulting in a total of 22 unique preferred potential analogs (Table_Apx E-3).

Table_Apx E-2. Potential Analogs Identified for Read-Across Analysis (No Particular Order)

Chemical Name	CASRN
1-Bromo*o*-4-chlorobenzene	106-39-8
4-Chlorotoluene	106-43-4
p-Dichlorobenzene	106-46-7
Benzene, bromotrichloro-	107103-78-6
Benzene, 1-bromo*o*-2-fluoro-	1072-85-1
Benzene, 1-bromo*o*-3-chloro-	108-37-2
3-Chlorotoluene	108-41-8
1,3,5-Trichlorobenzene	108-70-3
Chlorobenzene	108-90-7
2,6-Dichlorotoluene	118-69-4
1-Bromo*o*-2,4-dichlorobenzene	1193-72-2
Trichlorobenzene (mixed isomers)	12002-48-1
1,2,4-Trichlorobenzene	120-82-1
Tetrachlorobenzene (mixed isomers)	12408-10-5
Benzene, 1-chloro-3,5-difluoro-	1435-43-4
1-Chloro-2,4-difluorobenzene	1435-44-5
Benzene, 1,3-dichloro-5-fluoro-	1435-46-7

Chemical Name	CASRN
Benzene, 2,4-dichloro-1-fluoro-	1435-48-9
1,2-Dichloro-4-fluorobenzene	1435-49-0
Benzene, 2-bromo-1,4-dichloro-	1435-50-3
Benzene, 1,3-dibromo-5-chloro-	14862-52-3
Benzene, 1,2,3-trifluoro-	1489-53-8
3-Chloro-4-fluorotoluene	1513-25-3
5-Bromo-1,3-dichloro-2-fluorobenzene	17318-08-0
o- or *p*-Dichlorobenzene (mixed isomers)	179601-82-2
Benzene, 4-bromo-1,2-dichloro-	18282-59-2
1-Bromo-2,6-dichlorobenzene	19393-92-1
2,5-Dichlorotoluene	19398-61-9
Benzene, 1-bromo-3,5-dichloro-	19752-55-7
1-Bromo-4-chloro-2-fluorobenzene	1996-29-8
2-Bromo-1-chloro-4-fluorobenzene	201849-15-2
1-Bromo-2,5-dichloro-3-fluorobenzene	202865-57-4
2,3,6-Trichlorotoluene	2077-46-5
3,4,5-Trichlorotoluene	21472-86-6
1,3-Dichloro-2-fluorobenzene	2268-05-5
Benzene, 1-chloro-2,4,5-trifluoro-	2367-78-4
Benzene, 1,3-dichloro-2,5-difluoro-	2367-80-8
1-Chloro-2,5-difluorobenzene	2367-91-1
1,3,5-Trichloro-2-methylbenzene	23749-65-7
Chlorotoluene (mixed isomers)	25168-05-2
3,5-Dichlorotoluene	25186-47-4
Dichlorobenzene (mixed isomers)	25321-22-6
Benzene, difluoro- (mixed isomers)	27858-05-5
Bromochlorobenzene (mixed isomers)	28906-38-9
1-Bromo-2,4,5-trichlorobenzene	29682-44-8
Dichloromethylbenzene (mixed isomers)	29797-40-8
Trichloromethylbenzene (mixed isomers)	30583-33-6
Benzene, 2,3,5-trichloro-1-fluoro-	3107-20-8
Benzene, 1,2-dichloro-3-methyl-	32768-54-0
2-Chloro-5-fluorotoluene	33406-96-1
1-Chloro-2-fluorobenzene	348-51-6
Benzene, 1,4-dichloro-2-fluoro-	348-59-4
Benzene, 1-chloro-4-fluoro-	352-33-0
Benzene, 1,3,5-trichloro-2-fluoro-	36556-33-9
Benzene, 1,2,3-trichloro-4-fluoro-	36556-36-2
Benzene, 1,3-dichloro-2,4-difluoro-	36556-37-3
Benzene, 1,2-dichloro-3,4-difluoro-	36556-39-5
Benzene, 1-chloro-2,3,4-trifluoro-	36556-42-0

Chemical Name	CASRN
2,3-Dichloro-1-fluorobenzene	36556-50-0
Benzene, 2,3-dichloro-1,4-difluoro-	36556-54-4
o-Difluorobenzene	367-11-3
1,2,4-Trifluorobenzene	367-23-7
m-Difluorobenzene	372-18-9
1,3,5-Trifluorobenzene	372-38-3
Benzene, 2-chloro-1,3-difluoro-	38361-37-4
Benzene, 2-chloro-1,3,4-trifluoro-	39153-73-6
Sodium 3,4-dichlorophenolate	39975-26-3
1,2,4-Trichloro-5-fluorobenzene	400-04-4
Benzene, 1,4-dichloro-2,5-difluoro-	400-05-5
2-Chloro-6-fluorotoluene	443-83-4
5-Chloro-2-fluorotoluene	452-66-4
2-Chloro-4-fluorotoluene	452-73-3
4-Chloro-2-fluorotoluene	452-75-5
Phenol, 3,4-dichloro-, ion(1-)	45670-76-6
Fluorobenzene	462-06-6
p-Difluorobenzene	540-36-3
m-Dichlorobenzene	541-73-1
4-Chloro-3-fluorotoluene	5527-94-6
1-Bromo-2,3-dichlorobenzene	56961-77-4
1,2,5-Trichloro-3-methylbenzene	56961-86-5
o-Dibromobenzene	583-53-9
1-Bromo-2-chloro-4,5-difluorobenzene	59447-06-2
4-Bromo-1-chloro-2-fluorobenzene	60811-18-9
Benzene, 1,2-dibromo-4-chloro-	60956-24-3
Benzene, 1-chloro-3-fluoro-	625-98-9
1,2,3,4-Tetrachlorobenzene	634-66-2
1,2,3,5-Tetrachlorobenzene	634-90-2
2,4,5-Trichlorotoluene	6639-30-1
1-Bromo-2-chlorobenzene	694-80-4
1-Chloro-3,4-difluorobenzene	696-02-6
2,3,4-Trichlorotoluene	7359-72-0
1-Bromo-2,3,5-trichlorobenzene	81067-38-1
2-Chloro-3-fluorotoluene	85089-31-2
1,2,3-Trichlorobenzene	87-61-6
3-Chloro-5-fluorotoluene	93857-90-0
2-Chlorotoluene	95-49-8
2,4-Dichlorotoluene	95-73-8
3,4-Dichlorotoluene	95-75-0
3,4-Dichlorophenol	95-77-2

Chemical Name	CASRN
1,2,4,5-Tetrachlorobenzene	95-94-3

Table_Apx E-3. Preferred Potential Analogs Identified for Read-Across Analysis (Sorted by Preference)[a]

Chemical Name	CASRN
Chlorinated Benzenes with One to Three Chlorines and Potential Metabolites	
_p_-Dichlorobenzene	**106-46-7**
_m_-Dichlorobenzene	541-73-1
_o_- or _p_-Dichlorobenzene (mixed isomers)	179601-82-2
Dichlorobenzene (mixed isomers)	25321-22-6
3,4-Dichlorophenol	95-77-2
3,4-Dichlorophenol sodium salt	39975-26-3
Phenol, 3,4-dichloro-, ion(1-)	45670-76-6
Chlorobenzene	**108-90-7**
1,2,4-Trichlorobenzene	**120-82-1**
1,2,3-Trichlorobenzene	**87-61-6**
1,3,5-Trichlorobenzene	**108-70-3**
Trichlorobenzene (mixed isomers)	12002-48-1
Halogenated Benzenes with One to Three Halogens (Chlorine/Bromine Preferred)	
1-Bromo-2-chlorobenzene	694-80-4
1-Chloro-2-fluorobenzene	348-51-6
_o_-Dibromobenzene	583-53-9
1-Bromo-2-fluorobenzene	1072-85-1
_o_-Difluorobenzene	367-11-3
Tetra-chlorinated Benzenes	
1,2,3,4-Tetrachlorobenzene	634-66-2
1,2,3,5-Tetrachlorobenzene	634-90-2
1,2,4,5-Tetrachlorobenzene	95-94-3
Tetrachlorobenzene (mixed isomers)	12408-10-5
Chlorinated Methylbenzenes	
2-Chlorotoluene	**95-49-8**
[a] Potential analogs with inhalation toxicity values are in bold font.	

E.3 Analogs with Inhalation Toxicity Values

Subchronic and chronic inhalation noncancer toxicity values for the preferred potential analogs were obtained from data sources including the U.S. EPA (IRIS, PPRTV assessments, and Regional Screening Levels [RSLs] for Chemical Contaminants at Superfund Sites), ATSDR, CalEPA OEHHA, Health Canada, Rijksinstituut voor Volksgezondheid en Milieu (RIVM), and Danish-Derived No-Effect Levels (DNELs). Six analogs with noncancer inhalation toxicity values were identified (_p_-dichlorobenzene, chlorobenzene, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, and 2-chlorotoluene). These toxicity values are

summarized in *Draft Candidate Analog and Toxicity Values to Support ReCAAP Analysis for o-Dichlorobenzene* (U.S. EPA, 2026m). In addition, searches were conducted for inhalation cancer slope factors and inhalation unit risk values in data sources including U.S. EPA IRIS and PPRTV, CalEPA (OEHHA), Health Canada, and other sources identified in the U.S. EPA CompTox Chemicals Dashboard (Version 2.6.0). One analog with quantitative inhalation cancer values was identified (*p*-dichlorobenzene).

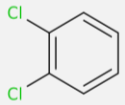
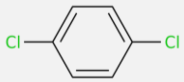
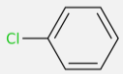
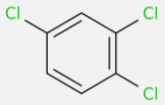
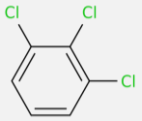
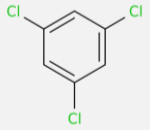
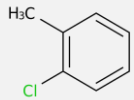
E.3.1 Physical and Chemical Properties

Table_Apx E-4 presents a comparison of the physical-chemical properties for *o*-dichlorobenzene and the six preferred potential analogs with inhalation toxicity values. For *o*- and *p*-dichlorobenzene, values were obtained from the U.S. EPA's systematic review for TSCA risk evaluations. For the other chemicals, values were obtained from U.S. EPA's CompTox Chemicals Dashboard. *o*-Dichlorobenzene and the six potential analogs are chlorinated benzene derivatives with one to three chlorines; one of the six potential analogs, 2-chlorotoluene, also has a methyl group.

- While *o*-dichlorobenzene and three potential analogs (1,2,4-trichlorobenzene, chlorobenzene, and 2-chlorotoluene) are liquids, *p*-dichlorobenzene and 1,2,3- and 1,3,5-trichlorobenzene are solids at room temperature.
- *o*-Dichlorobenzene and all analogs have moderate vapor pressure except chlorobenzene and 2-chlorotoluene, which have high vapor pressures.
- *o*-Dichlorobenzene and all analogs have moderate water solubilities ranging from 6.21 to 484 mg/L, with the value for *o*-dichlorobenzene falling in the middle of the range (156 mg/L).
- *o*-Dichlorobenzene has a log K_{ow} of 3.43. Despite structural differences, all analogs share relatively similar values, with 1,3,5-trichlorobenzene being the most hydrophobic (log K_{ow} = 4.19) and chlorobenzene being the least hydrophobic (log K_{ow} = 2.87).
- All these chemicals may volatilize from water surfaces given their Henry's Law constants.

In summary, *o*-dichlorobenzene and the six analogs exhibit similarities in water solubility and log K_{ow}. Some differences in vapor pressure were observed.

Table_Apx E-4. Physical and Chemical Properties of *o*-Dichlorobenzene and Preferred Potential Analogs with Inhalation Toxicity Values

Structure							
Name (CASRN)	***o*-Dichlorobenzene (CASRN 95-50-1)[a]**	***p*-Dichlorobenzene (CASRN 106-46-7)[a]**	**Chlorobenzene (CASRN 108-90-7)[b]**	**1,2,4-Trichlorobenzene (CASRN 120-82-1)[b]**	**1,2,3-Trichlorobenzene (CASRN 87-61-6)[b]**	**1,3,5-Trichlorobenzene (CASRN 108-70-3)[b]**	**2-Chlorotoluene (CASRN 95-49-8)[b]**
Molecular formula	$C_6H_4Cl_2$	$C_6H_4Cl_2$	C_6H_5Cl	$C_6H_3Cl_3$	$C_6H_3Cl_3$	$C_6H_3Cl_3$	C_7H_7Cl
Molecular weight (g/mol)	147.00	147.00	112.56	181.44	181.44	181.44	126.58
Physical form	Liquid	Solid	Liquid	Liquid	Solid	Solid	Liquid
Melting point (°C)	-17.03	53.1	-45.2	17	53.3	63.8	-35.6
Boiling point (°C)	180.2	174	132	213	219	208	159
Vapor pressure (mm Hg)	1	1	12	0.460	0.210	0.240	3.43
Water solubility (mg/L)	156	81.3	484 (reported as 4.30×10^{-3} mol/L)	46.4 (reported as 2.56×10^{-4} mol/L)	20.1 (reported as 1.11×10^{-4} mol/L)	6.21 (reported as 3.42×10^{-5} mol/L)	206 (reported as 1.63×10^{-3} mol/L)
Octanol/water partition coefficient (log K_{ow})	3.43	3.23±0.03	2.87	4.00	4.08	4.19	3.42
Henry's Law constant ($atm\cdot m^3/mol$ at 25 °C)	1.92×10^{-3}	2.41×10^{-3}	3.11×10^{-3}	1.42×10^{-3}	1.25×10^{-3}	1.89×10^{-3}	3.57×10^{-3}

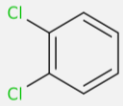
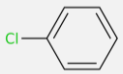
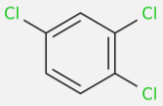
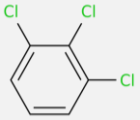
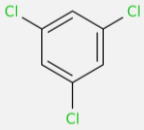
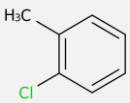
[a] Values obtained from the U.S. EPA's systematic review.
[b] Average experimental value reported in CompTox Chemicals Dashboard Version 2.6.0; data collected 22 September 2025.

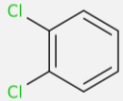
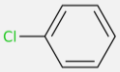
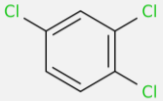
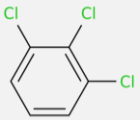
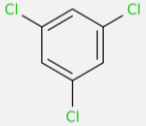
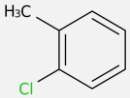
E.3.2 Absorption, Distribution, Metabolism, and Excretion

Toxicokinetic properties for *o*-dichlorobenzene and the six read-across analogs are summarized in Table_Apx E-2. Studies evaluating the rate and extent of absorption by the inhalation route are limited. Rapid absorption of *p*-dichlorobenzene and chlorobenzene was demonstrated by detection in blood and tissues of rats exposed by inhalation (ATSDR, 2020, 2006; U.S. EPA, 2006a); no data are available for absorption following inhalation for the other compounds. Experimental animal studies for *o*-dichlorobenzene and each of the read-across analogs (except chlorobenzene) showed rapid and extensive absorption from the gastrointestinal tract (ATSDR, 2014; U.S. EPA, 2010a; ATSDR, 2006; U.S. EPA, 2006a). Chlorobenzene is also readily absorbed via oral exposure in animals; however, data are too limited to determine the rate and extent of absorption (ATSDR, 2020). Once absorbed, widespread distribution to tissues was reported in experimental animal studies for *o*-dichlorobenzene, *p*-dichlorobenzene, chlorobenzene, and the trichlorobenzene analogs, with the highest concentration measured in fat (ATSDR, 2020, 2014; U.S. EPA, 2010a; ATSDR, 2006). No distribution data were located for 2-chlorotoluene (U.S. EPA, 2010a). Dichlorobenzene and trichlorobenzene compounds have been detected in human breast milk and adipose tissue (ATSDR, 2014; U.S. EPA, 2010a; ATSDR, 2006). No data were available on the presence of chlorobenzene or 2-chlorotoluene in human breast milk or adipose tissue (ATSDR, 2020; U.S. EPA, 2010a).

Toxicokinetic studies in animals and *in vitro* experiments demonstrate that chlorobenzene, dichlorobenzenes, and trichlorobenzenes are initially oxidized by CYP to form epoxides that are further metabolized to phenols, catechols, and/or quinones (ATSDR, 2020, 2014; U.S. EPA, 2010a; ATSDR, 2006). Dihydrodiol metabolites were also produced during metabolism of *o*-dichlorobenzene and chlorobenzene (ATSDR, 2020, 2006; U.S. EPA, 2006a). In contrast, 2-chlorotoluene undergoes methyl group oxidation to form 2-chlorobenzyl alcohol and 2-chlorobenzoic acid (U.S. EPA, 2010a). For *o*-dichlorobenzene and all read-across analogs, metabolites conjugated with GSH, sulfate, glucuronide, and/or glycine are rapidly excreted primarily in the urine with smaller amounts detected in feces and/or expired air (ATSDR, 2020, 2014; U.S. EPA, 2010a; ATSDR, 2006; U.S. EPA, 2006a).

Table_Apx E-5. Summary of Absorption, Distribution, Metabolism, and Excretion (ADME) Data for *o*-Dichlorobenzene and Read-Across Analogs

Structure							
Name (CASRN)	***o*-Dichlorobenzene (CASRN 95-50-1)**	***p*-Dichlorobenzene (CASRN 106-46-7)**	**Chlorobenzene (CASRN 108-90-7)**	**1,2,4-Trichlorobenzene (CASRN 120-82-1)**	**1,2,3-Trichlorobenzene (CASRN 87-61-6)**	**1,3,5-Trichlorobenzene (CASRN 108-70-3)**	**2-Chlorotoluene (CASRN 95-49-8)**
Absorption							
Rate and extent of inhalation absorption	No data	Rapid; no data on extent	Rapid and extensive	No data	No data	No data	No data
Rate and extent of oral absorption	Rapid and extensive	Rapid and extensive	Readily absorbed; limited data on rate and extent	Rapid and extensive	Rapid and extensive	Rapid and extensive	Rapid and extensive
Distribution							
Extent of distribution	Widespread distribution; highest concentration in adipose tissue	Widespread distribution; highest concentration in adipose tissue	Widespread distribution; highest concentration in adipose tissue	Widespread distribution; highest concentration in adipose tissue	Widespread distribution	Widespread distribution; highest concentration in adipose tissue	No data
Metabolism							
Pathways and enzymes	Ring oxidation to epoxides; GSH, sulfate, glucuronide conjugation	Ring oxidation to epoxides; GSH, sulfate, glucuronide conjugation	Ring oxidation to epoxides; GSH, sulfate, glucuronide conjugation	Ring oxidation to epoxides; GSH, sulfate, glucuronide conjugation	Ring oxidation to epoxides; sulfate and glucuronide conjugation	Ring oxidation to epoxides; sulfate and glucuronide conjugation	Methyl group oxidation and GSH, glucuronide, sulfate, and glycine conjugation
Metabolites	Phenols, dihydrodiols, catechols, quinones	Phenols, catechols, quinones	Phenols, dihydrodiols, catechols, quinones	Phenols, catechols, quinones	Phenols, catechols	Phenols	2-Chlorobenzyl alcohol, 2-chlorobenzoic acid

Structure							
Name (CASRN)	***o*-Dichlorobenzene (CASRN 95-50-1)**	***p*-Dichlorobenzene (CASRN 106-46-7)**	**Chlorobenzene (CASRN 108-90-7)**	**1,2,4-Trichlorobenzene (CASRN 120-82-1)**	**1,2,3-Trichlorobenzene (CASRN 87-61-6)**	**1,3,5-Trichlorobenzene (CASRN 108-70-3)**	**2-Chlorotoluene (CASRN 95-49-8)**
Excretion							
Route of excretion	Primarily urine, minor amount in feces	Primarily urine, minor amount in feces	Primarily urine, minor amounts in feces and expired air	Primarily urine, minor amounts in feces and expired air	Primarily urine, minor amount in feces	Primarily urine, minor amount in feces	Primarily urine, minor amounts in feces and expired air
Rate of excretion	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid
References	ATSDR (2006); U.S. EPA (2006a)	ATSDR (2006); U.S. EPA (2006a)	ATSDR (2020)	ATSDR (2014)	ATSDR (2014)	ATSDR (2014)	U.S. EPA (2010a)

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Species differences in metabolism have been noted for *o*- and *p*-dichlorobenzene. In microsomal studies, the overall conversion of *o*- and *p*-dichlorobenzene and the production of covalently bound reactive metabolites were greatest in mice compared to rats and humans (Nedelcheva et al., 1998; Hissink et al., 1997b; Hissink et al., 1996a). CYP2E1 is the primary isozyme involved in *o*- and *p*-dichlorobenzene metabolism in humans (Nedelcheva et al., 1998; Hissink et al., 1997b; Hissink et al., 1996a; Hissink et al., 1996c; Bogaards et al., 1995) and this isozyme is also involved in metabolism of both compounds in rats and mice (Nedelcheva et al., 1998; Hissink et al., 1997a). CYP2B also contributes to metabolism of *o*- and *p*-dichlorobenzene in rats and *o*-dichlorobenzene (with less certain evidence for *p*-dichlorobenzene) in mice (Hissink et al., 1997b; Hissink et al., 1996a; den Besten et al., 1992; Colacci et al., 1990).

In summary, toxicokinetic comparisons indicate that *o*-dichlorobenzene and the read-across analogs share common absorption, distribution, metabolism, and excretion properties except for 2-chlorotoluene, which is metabolized via methyl group oxidation instead of ring oxidation.

Appendix F GENE EXPRESSION BIOMARKER ANALYSIS OF RODENT LIVERS TREATED WITH *p*-DICHLOROBENZENE

Evidence shows that application of gene expression biomarker panels in short-term rodent exposure studies can identify both tumorigenic hazard as well as dose levels that would result in induction of liver tumors in chronic studies (Corton et al., 2022). Mouse liver biomarkers have been characterized to predict MIEs associated with mouse liver tumor induction. These efforts capitalized on available microarray data from chemically treated wild-type and transcription factor-null mice allowing for the identification of well-defined mechanistic gene sets. The gene expression biomarkers include those that predict activation of the receptors aryl hydrocarbon receptor (AhR) (Oshida et al., 2015b), CAR (Oshida et al., 2015a), and peroxisome proliferator-activated receptor alpha (PPARα) (Oshida et al., 2015c). In addition, biomarkers have been characterized which predict increases in oxidative stress by examining Nrf2 activation (Rooney et al., 2019; Rooney et al., 2018b) and which predict increases in cell proliferation (Corton et al., 2024). Most of these biomarkers have predictive accuracies greater than 91%. The cell proliferation biomarker can be used to predict liver cell proliferation in the mouse and rat, but the predictive accuracy has not been determined (Corton et al., 2024). These biomarkers have been applied to sets of chemicals to identify the most likely adverse outcome pathway (AOP) responsible for mouse liver tumors (Peffer et al., 2018; Rooney et al., 2017) or to understand the relationships between exposure and hazard (Rosen et al., 2017).

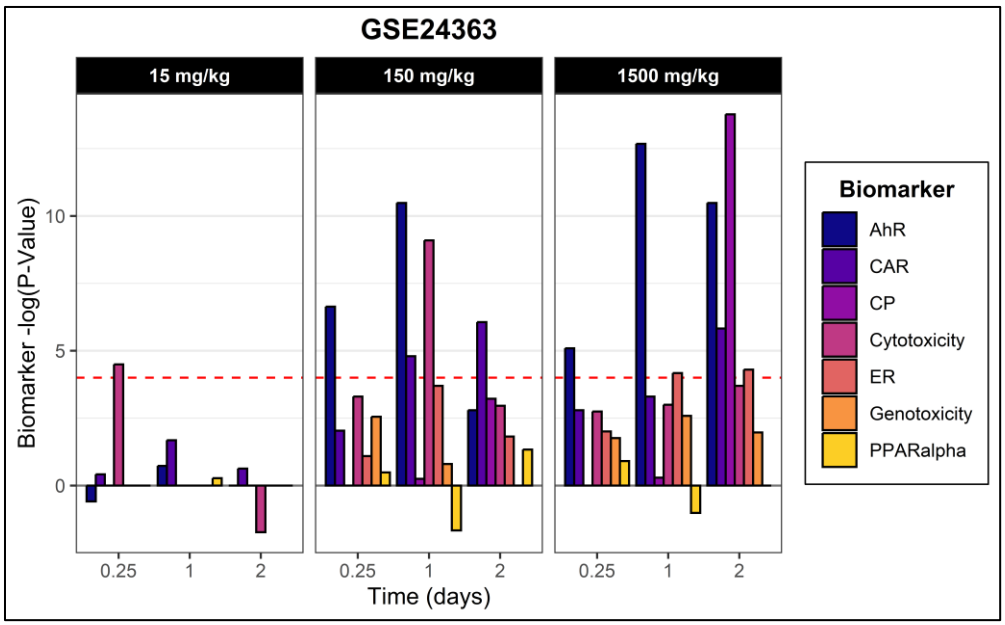
Several studies have described biomarkers that predict liver cancer MIEs and tumorigenic doses in the rat liver. In two studies, a set of six transcriptomic biomarkers were characterized that predict the major MIEs in AOPs by which chemicals cause liver tumors in rats (Corton et al., 2020; Rooney et al., 2018a). These biomarkers predict genotoxicity, cytotoxicity, and activation of AhR, CAR, ER, and PPARα. Each of the biomarkers was built using a set of gene expression profiles from the livers of rats treated with known inducers of the respective receptor, DNA damage, or cytotoxicity. The biomarkers predict the MIE of a test set of chemicals with excellent accuracies (91%–97%) across a wide range of doses and times of exposure. The activation levels of the MIEs can be used to predict potential tumorigenesis in rat livers even after short-term exposures (Ledbetter et al., 2024; Hill et al., 2020; Lewis et al., 2020; Qin et al., 2019). These studies required careful annotation of the known liver tumorigenic outcomes of each chemical-dose combination derived from two-year bioassay data archived in a number of databases such as the Lhasa database (https://carcdb.lhasalimited.org/, accessed July 10, 2025; based on the Carcinogenicity Potency Database). The activation levels of 6 MIEs associated with liver tumor induction had a predictive accuracy of 91%–97% depending on the activation levels used (Hill et al., 2020), with low false negative rates—a highly desirable characteristic for regulatory determination of hazard. In follow-up studies (Lewis et al., 2020), an analysis of approximately 50 chemicals profiled in rat liver from an independent dataset yielded approximately 90% predictive accuracy.

The genes altered by chemical exposure are compared to each biomarker in a pair-wise fashion using the Running Fisher test resulting in a correlation p-value that can be used to determine activation (Kupershmidt et al., 2010). A -Log(p-value) greater than or equal to four is considered a significant activation/increase (see individual studies for details on how accuracy was determined).

A review of the literature, Gene Expression Omnibus (GEO), and studies archived in BaseSpace Correlation Engine identified one rat study ((Consortium, 2010; Popovici et al., 2010; Shi, 2010); GSE24363) in which animals were treated with *o*-dichlorobenzene (15, 150, 1,500 mg/kg) for 0.25, 1 or 2 days. Full genome gene expression profiling was carried out on the livers using Affymetrix rat 230 2.0

arrays. Each of the treatment conditions were assessed for MIE activation using 7 gene expression biomarkers described above.

After 1,2-dichlorobenzene treatment, cytotoxicity was increased at 15 mg/kg at 0.25 days and at 150 mg/kg at 1 day (Figure_Apx F-1). Cytotoxicity approached significance at 1500 mg/kg at 2 days. AhR was activated at 150 mg/kg (0.25 days, 1 day) and 1500 mg/kg (all three time points). CAR was activated at 150 mg/kg at 1 and 2 days and at 1500 mg/kg at 2 days. ER was activated only at the 1500 mg/kg dose level (1 and 2 days). Cell proliferation was increased at 1500 mg/kg at 2 days. There was no activation of PPARα or genotoxicity at any dose or time point.



Figure_Apx F-1. Biomarker analysis of the livers of rats exposed to *o*-dichlorobenzene (GSE24363)

Appendix G RUBRIC FOR WEIGHT OF SCIENTIFIC EVIDENCE – ENVIRONMENTAL HAZARD

The weight of scientific evidence fundamentally means that the evidence is weighed (*i.e.,* ranked) and weighted (*i.e.,* a piece or set of evidence or uncertainty may have more importance or influence in the result than another). Based on the weight of scientific evidence and uncertainties, a confidence statement was developed that qualitatively ranks (*i.e.,* robust, moderate, slight, or indeterminate) the confidence in the hazard threshold. The qualitative confidence levels are described below.

The evidence considerations and criteria detailed within *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021) which guides the application of strength-of-evidence judgments for environmental hazard effect within a given evidence stream.

EPA used the strength-of-evidence and uncertainties from (U.S. EPA, 2021) for the hazard assessment to qualitatively rank the overall confidence rating for environmental hazard (Table_Apx G-2). Confidence levels of robust (+ + +), moderate (+ +), slight (+), or indeterminant are assigned for each evidence property that corresponds to the evidence considerations (U.S. EPA, 2021). The rank of the *Quality of the Database* consideration is based on the systematic review overall quality determination (High, Medium, or Low) for studies used to calculate the hazard threshold, and whether there are data gaps in the toxicity data set. Another consideration in the *Quality of the Database* is the risk of bias (*i.e.,* how representative is the study to ecologically relevant endpoints). Additionally, because of the importance of the studies used for deriving hazard thresholds, the *Quality of the Database* consideration may have greater weight than the other individual considerations. The high, medium, and low systematic review overall quality determination ranks correspond to the evidence table ranks of robust (+ + +), moderate (+ +), or slight (+), respectively. The evidence considerations are weighted based on professional judgment to obtain the overall confidence for each hazard threshold. In other words, the weights of each evidence property relative to the other properties are dependent on the specifics of the weight of scientific evidence and uncertainties that are described in the narrative and may or may not be equal. Therefore, the overall score is not necessarily a mean or defaulted to the lowest score. The confidence levels and uncertainty type examples are described below.

G.1 Confidence Levels

- Robust (+ + +) confidence suggests thorough understanding of the scientific evidence and uncertainties. The supporting weight of scientific evidence outweighs the uncertainties to the point where it is unlikely that the uncertainties could have a significant effect on the exposure or hazard estimate.
- Moderate **(**+ +) confidence suggests some understanding of the scientific evidence and uncertainties. The supporting scientific evidence weighed against the uncertainties is reasonably adequate to characterize exposure or hazard estimates.
- Slight (+) confidence is assigned when the weight of scientific evidence may not be adequate to characterize the scenario, and when the assessor is making the best scientific assessment possible in the absence of complete information. There are additional uncertainties that may need to be considered.

G.2 Types of Uncertainties

The following uncertainties may be relevant to one or more of the weight of scientific evidence considerations listed above and will be integrated into that property's rank in the evidence table:

- *Scenario Uncertainty:* Uncertainty regarding missing or incomplete information needed to fully define the exposure and dose.
  - The sources of scenario uncertainty include descriptive errors, aggregation errors, errors in professional judgment, and incomplete analysis.
- *Parameter Uncertainty:* Uncertainty regarding some parameter.
  - Sources of parameter uncertainty include measurement errors, sampling errors, variability, and use of generic or surrogate data.
- *Model Uncertainty:* Uncertainty regarding gaps in scientific theory required to make predictions based on causal inferences.
  - Modeling assumptions may be simplified representations of reality.

Table_Apx G-1 summarizes the weight of scientific evidence and uncertainties, while increasing transparency on how EPA arrived at the overall confidence level for each exposure hazard threshold. Symbols are used to provide a visual overview of the confidence in the body of evidence, while de-emphasizing an individual ranking that may give the impression that ranks are cumulative (*e.g.,* ranks of different categories may have different weights).

Table_Apx G-1. *o*-Dichlorobenzene Evidence Table Summarizing the Overall Confidence Derived from Hazard Thresholds

Types of Evidence	Quality of the Database	Consistency	Strength and Precision	Biological Gradient/Dose-Response	Relevance	Hazard Confidence
Aquatic						
Acute Aquatic Vertebrate and Invertebrate (SSD)	+++	+++	+++	+++	+++	Robust
Chronic Aquatic Vertebrates	++	++	++	++	++	Moderate
Chronic Aquatic Invertebrates	++	++	++	++	++	Moderate
Aquatic Plants & Algae	+++	+++	+++	+++	+++	Robust
Terrestrial Vertebrates	++	++	++	++	+	Moderate
Terrestrial Invertebrates	+	Not applicable	+	+	+	Slight
Terrestrial Plants	+	+	+	+	+	Slight

+++ Robust confidence suggests thorough understanding of scientific evidence and uncertainties. The supporting weight of scientific evidence outweighs the uncertainties to the point where it is unlikely that the uncertainties could have a significant effect on the hazard estimate.
++ Moderate confidence suggests some understanding of scientific evidence and uncertainties. The supporting scientific evidence weighed against the uncertainties is reasonably adequate to characterize hazard estimates.
+ Slight confidence is assigned when the weight of scientific evidence may not be adequate to characterize the scenario, and when the assessor is making the best scientific assessment possible in the absence of complete information. There are additional uncertainties that may need to be considered.

Table_Apx G-2. Considerations that Inform Evaluations of the Strength of the Evidence within an Evidence Stream (*i.e.*, Apical Endpoints, Mechanistic, or Field Studies)

Consideration	Increased Evidence Strength (of the Apical Endpoints, Mechanistic, or Field Studies Evidence)	Decreased Evidence Strength (of the Apical Endpoints, Mechanistic, or Field Studies Evidence)
The evidence considerations and criteria laid out here guide the application of strength-of-evidence judgments for an outcome or environmental hazard effect within a given evidence stream. Evidence integration or synthesis results that do not warrant an increase or decrease in evidence strength for a given consideration are considered "neutral" and are not described in this table (and, in general, are captured in the assessment-specific evidence profile tables).		
Quality of the database[a] (risk of bias)	• A large evidence base of *high*- or *medium*-quality studies increases strength. • Strength increases if relevant species are represented in a database.	• An evidence base of mostly *low*-quality studies decreases strength. • Strength also decreases if the database has data gaps for relevant species, *i.e.,* a trophic level that is not represented. • Decisions to increase strength for other considerations in this table should generally not be made if there are serious concerns for risk of bias; in other words, all the other considerations in this table are dependent upon the quality of the database.
Consistency	Similarity of findings for a given outcome (*e.g.,* of a similar magnitude, direction) across independent studies or experiments increases strength, particularly when consistency is observed across species, life stage, sex, wildlife populations, and across or within aquatic and terrestrial exposure pathways.	• Unexplained inconsistency (*i.e.,* conflicting evidence decreases strength, see U.S. EPA (2005)) • Strength should not be decreased if discrepant findings can be reasonably explained by study confidence conclusions; variation in population or species, sex, or life stage; frequency of exposure (*e.g.,* intermittent or continuous); exposure levels (low or high); or exposure duration.
Strength (effect magnitude) and precision	• Evidence of a large magnitude effect (considered either within or across studies) can increase strength. • Effects of a concerning rarity or severity can also increase strength, even if they are of a small magnitude. • Precise results from individual studies or across the set of studies increases strength, noting that biological significance is prioritized over statistical significance. • Use of probabilistic model (*e.g.,* Web-ICE, SSD) may increase strength.	Strength may be decreased if effect sizes that are small in magnitude are concluded not to be biologically significant, or if there are only a few studies with imprecise results.
Biological gradient/dose-response	• Evidence of dose-response increases strength. • Dose-response may be demonstrated across studies or within studies and it can be dose- or duration-dependent.	• A lack of dose-response when expected based on biological understanding and having a wide range of doses/exposures evaluated in the evidence base can decrease strength.

Consideration	Increased Evidence Strength (of the Apical Endpoints, Mechanistic, or Field Studies Evidence)	Decreased Evidence Strength (of the Apical Endpoints, Mechanistic, or Field Studies Evidence)
	• Dose response may not be a monotonic dose-response (monotonicity should not necessarily be expected, *e.g.,* different outcomes may be expected at low vs. high doses due to activation of different mechanistic pathways or induction of systemic toxicity at very high doses). • Decreases in a response after cessation of exposure (*e.g.,* return to baseline fecundity) also may increase strength by increasing certainty in a relationship between exposure and outcome (this particularly applicable to field studies).	• In experimental studies, strength may be decreased when effects resolve under certain experimental conditions (*e.g.,* rapid reversibility after removal of exposure). • However, many reversible effects are of high concern. Deciding between these situations is informed by factors such as the toxicokinetics of the chemical and the conditions of exposure, see (U.S. EPA, 1998), endpoint severity, judgments regarding the potential for delayed or secondary effects, as well as the exposure context focus of the assessment (*e.g.,* addressing intermittent or short-term exposures). • In rare cases, and typically only in toxicology studies, the magnitude of effects at a given exposure level might decrease with longer exposures (*e.g.,* due to tolerance or acclimation). • Like the discussion of reversibility above, a decision about whether this decreases evidence strength depends on the exposure context focus of the assessment and other factors. • If the data are not adequate to evaluate a dose-response pattern, then strength is neither increased nor decreased.
Biological relevance	Effects observed in different populations or representative species suggesting that the effect is likely relevant to the population or representative species of interest (*e.g.,* correspondence among the taxa, life stages, and processes measured or observed and the assessment endpoint).	An effect observed only in a specific population or species without a clear analogy to the population or representative species of interest decreases strength.
Physical/chemical relevance	Correspondence between the substance tested and the substance constituting the stressor of concern.	The substance tested is an analog of the chemical of interest or a mixture of chemicals which include other chemicals besides the chemical of interest.
Environmental relevance	Correspondence between test conditions and conditions in the region of concern.	The test is conducted using conditions that would not occur in the environment.

[a] Database refers to the entire data set of studies integrated in the environmental hazard assessment and used to inform the strength of the evidence. In this context, database does *not* refer to a computer database that stores aggregations of data records such as the ECOTOX Knowledgebase.

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Appendix H SPECIES SENSITIVITY DISTRIBUTION FOR ACUTE AQUATIC HAZARD

The SSD Toolbox is a resource that can fit SSDs to environmental hazard data (Etterson, 2020). It runs on Matlab 2018b (9.5) for Windows 64 bit. For this draft *o*-dichlorobenzene risk evaluation, EPA created one SSD with the SSD Toolbox Version 1.0 to evaluate acute aquatic vertebrate and invertebrate toxicity. The use of this probabilistic approach increases confidence in the hazard threshold identification as it is a more data-driven way of accounting for uncertainty. For the acute SSD, acute exposure hazard data for aquatic vertebrates and invertebrates were curated to prioritize study quality and to assure comparability between toxicity values. For example, the empirical data set included only LC50s for high and medium quality acute duration assays (96-hour) that measured mortality for aquatic vertebrates and invertebrates. Table_Apx H-1 shows the empirical data that were used in the SSD. To further improve the fit and representativeness of the SSD, Web-ICE acute toxicity predictions for additional species were incorporated (Table_Apx H-2).

With this data set, the SSD Toolbox was used to apply a variety of algorithms to fit and visualize SSDs with different distributions. An HC_{05} is calculated for each. The SSD Toolbox's output contained several methods for choosing an appropriate distribution and fitting method, including goodness-of-fit and standard error (Burnham and Anderson, 2002). All p-values were acceptable for distributions fit with the Metropolis-Hastings fitting method (a Bayesian method; p-values >0.025 and <0.975). The Normal and Weibull distributions fit with the Maximum Likelihood method also had acceptable p-values above 0.05. Visual inspections of the data were also used to assess goodness-of-fit. For the Q-Q plot, the horizontal axis gives the empirical quantiles while the vertical axis gives the predicted quantiles (from the fitted distribution). The Q-Q plot demonstrates a good model fit when the data points are near the line across the data distribution. Q-Q plots were visually used to assess the goodness-of-fit for the distributions (Figure_Apx H-1) with the Logistic distribution fit with the Metropolis-Hastings method demonstrating the best fit near the low end of the distribution, which is the region from which the HC_{05} is derived. The results for this model (Table_Apx H-2) predicted 5% of the species (HC_{05}) to have their LC50s exceeded at 0.5956 mg/L (0.3799 to 0.8352 mg/L 95% CI).

Table_Apx H-1. SSD Model Input for Acute Exposure Toxicity in Aquatic Vertebrates and Invertebrates – Empirical Data

Species	Common Name	Acute Toxicity Value LC50 (mg/L)	Citation(s)
Oncorhynchus mykiss	Rainbow trout	1.61	U.S. EPA (1984)
Oncorhynchus mykiss	Rainbow trout	1.67	Dow Chemical (1974)
Oncorhynchus mykiss	Rainbow trout	1.58	Call et al. (1983)
Pimephales promelas	Fathead minnow	9.47	Geiger et al. (1986)
Pimephales promelas	Fathead minnow	6.03	Sum et al. (1993)
Poecilia reticulata	Guppy	4.79	Sum et al. (1993)
Eurytemora affinis	Copepod	15.9	Lindley et al. (1999)
Acartia clausi	Copepod	2.10	Lindley et al. (1999)

Table_Apx H-2. SSD Model Input for Acute Exposure Toxicity in Aquatic Organisms – Web-ICE Data

Species	Common Name	Acute Toxicity Value LC50 (mg/L)
Atherix variegata	Snipefly	7.7
Thamnocephalus platyurus	Beaver-tail fairy shrimp	0.53
Acartia tonsa	Copepod	0.61
Acipenser brevirostrum	Shortnose sturgeon	1.4
Allorchestes compressa	Amphipod	2.7
Allorchestes compressa	Amphipod	3.1
Amblema plicata	Threeridge	0.18
Ameiurus melas	Black bullhead	11
Americamysis bigelowi	Mysid	0.91
Americamysis bigelowi	Mysid	0.92
Bidyanus bidyanus	Silver perch	2.9
Caecidotea brevicauda	Isopod	0.80
Caecidotea brevicauda	Isopod	0.81
Carassius auratus	Goldfish	5.4
Carassius auratus	Goldfish	9.5
Catostomus commersonii	White sucker	12
Cirrhinus mrigala	Mrigal carp	5.1
Crangon crangon	Common shrimp	8.2
Crangon crangon	Common shrimp	5.6
Crassostrea virginica	Eastern oyster	2.7
Cyprinodon variegatus	Sheepshead minnow	2.8
Cyprinodon variegatus	Sheepshead minnow	4.8
Cyprinodon variegatus	Sheepshead minnow	2.2
Cyprinus carpio	Common carp	3.2
Cyprinus carpio	Common carp	8.0
Danio rerio	Zebrafish	2.0
Danio rerio	Zebrafish	6.9
Danio rerio-embryo	Zebrafish-embryo	4.4
Danio rerio-embryo	Zebrafish-embryo	9.8
Daphnia magna	Daphnid	0.56
Drunella grandis	Mayfly	7.4
Duttaphrynus melanostictus	Asian common toad	16
Esox lucius	Northern pike	1.1
Gammarus minus	Amphipod	21
Gibelion catla	Catla	7.6
Gila elegans	Bonytail	4.6

Species	Common Name	Acute Toxicity Value LC50 (mg/L)
Ictalurus punctatus	Channel catfish	2.8
Ictalurus punctatus	Channel catfish	7.1
Ictalurus punctatus	Channel catfish	11
Jordanella floridae	Flagfish	4.2
Jordanella floridae	Flagfish	2.9
Labeo rohita	Rohu	13
Lampsilis siliquoidea	Fatmucket	1.7
Lepidocephalichthys guntea	Peppered loach	5.6
Lepomis cyanellus	Green sunfish	3.1
Lepomis macrochirus	Bluegill	2.0
Lepomis macrochirus	Bluegill	4.2
Lepomis macrochirus	Bluegill	2.8
Limnodrilus hoffmeisteri	Oligochaete	7.4
Lithobates catesbeianus	Bullfrog	4.3
Lithobates catesbeianus	Bullfrog	8.2
Lithobates pipiens	Northern leopard frog	7.7
Lithobates sphenocephalus	Southern leopard frog	6.9
Lophopodella carteri	Bryozoan	1.0
Lumbriculus variegatus	Oligochaete	8.5
Lymnaea stagnalis	Swamp lymnaea	1.5
Menidia beryllina	Inland silverside	1.1
Menidia menidia	Atlantic silverside	0.71
Menidia menidia	Atlantic silverside	9.7
Micropterus salmoides	Largemouth bass	1.2
Micropterus salmoides	Largemouth bass	2.4
Morone saxatilis	Striped bass	2.1
Notropis mekistocholas	Cape Fear shiner	3.4
Oncorhynchus clarkii	Cutthroat trout	1.6
Oncorhynchus clarkii	Cutthroat trout	2.5
Oncorhynchus clarkii	Cutthroat trout	2.6
Oncorhynchus gilae	Apache trout	1.5
Oncorhynchus kisutch	Coho salmon	2.3
Oncorhynchus kisutch	Coho salmon	2.0
Oncorhynchus nerka	Sockeye salmon	2.1
Oncorhynchus tshawytscha	Chinook salmon	1.9
Oncorhynchus tshawytscha	Chinook salmon	1.9
Oreochromis mossambicus	Mozambique tilapia	5.1

Species	Common Name	Acute Toxicity Value LC50 (mg/L)
Oreochromis mossambicus	Mozambique tilapia	12
Oryzias latipes	Medaka	8.6
Oryzias latipes	Medaka	4.9
Paratanytarsus dissimilis	Midge	7.5
Paratanytarsus dissimilis	Midge	20
Perca flavescens	Yellow perch	1.5
Perca flavescens	Yellow perch	1.8
Physalaemus cuvieri	Cuvier's foam froglet	8.4
Physella gyrina	Tadpole physa	1.4
Ptychocheilus lucius	Colorado squawfish	6.9
Salmo salar	Atlantic salmon	1.5
Salmo trutta	Brown trout	1.7
Salvelinus fontinalis	Brook trout	1.6
Salvelinus fontinalis	Brook trout	1.9
Salvelinus fontinalis	Brook trout	1.3
Salvelinus namaycush	Lake trout	1.3
Salvelinus namaycush	Lake trout	1.1
Sander vitreus	Walleye	0.60
Tigriopus japonicus	Copepod	19
Tubifex tubifex	Oligochaete	8.4
Tubifex tubifex	Oligochaete	2.3
Utterbackia imbecillis	Paper pondshell	1.5
Xenopus laevis	African clawed frog	5.3
Xyrauchen texanus	Razorback sucker	2.6

Table_Apx H-3. SSD Model Predictions[a] for Acute Exposure Toxicity to Aquatic Organisms

Fitting Method	Distribution[b]	HC_{05} (mg/L)	P-value
Maximum Likelihood	Normal	0.6546	0.1638
	Logistic	0.6233	0.0150
	Triangular	0.3936	0.0040
	Gumbel	0.6377	0.0120
	Weibull	0.3940	0.0539
	Burr	0.5803	0.0080
Metropolis-Hastings	Normal	0.6230	0.2250
	Logistic	**0.5956**	**0.1790**
	Triangular	0.3696	0.9566
	Gumbel	0.6244	0.8916
	Weibull	0.4112	0.3652

Fitting Method	Distribution[b]	HC_{05} (mg/L)	P-value
	Burr	0.5436	0.4080

[a] The SSD was generated using SSD Toolbox v1.0.
[b] The model with the best model fit is bolded

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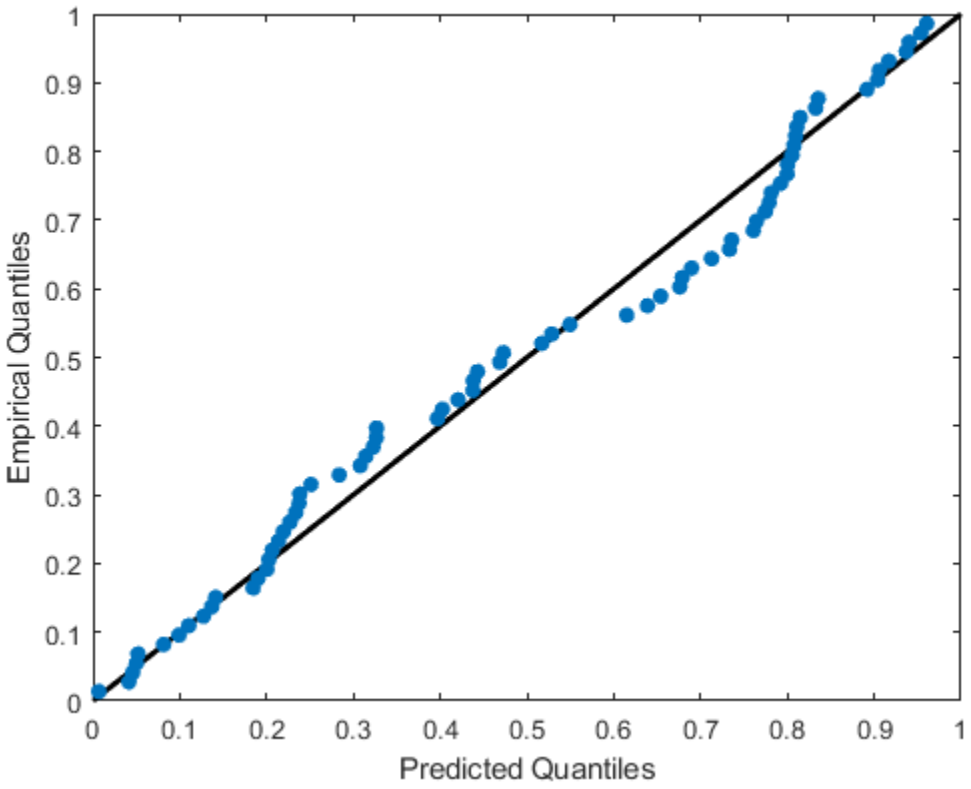


4862
4863 **Figure_Apx H-1. Q-Q Plot of Acute *o*-Dichlorobenzene Toxicity to**
4864 **Aquatic Vertebrates and Invertebrates with Metropolis-Hastings**
4865 **Method and Logistic Distribution**

Appendix I ECOSAR (version 2.2) Report *o*-Dichlorobenzene Predictive Model

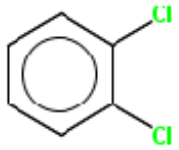
Created on Mar 4, 2025 1:27:43 PM

Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
95501	Benzene, 1,2-dichloro-	c(c(ccc1)Cl)(c1)Cl

Structure



Details	
Mol Wt	147
Selected LogKow	3.38
Selected Water Solubility (mg/L)	141
Selected Melting Point (°C)	-17.2
Estimated LogKow	3.28
Estimated Water Solubility (mg/L)	142.54
Measured LogKow	3.43
Measured Water Solubility (mg/L)	80
Measured Melting Point (°C)	-16.7

Class Results:	

Neutral Organics

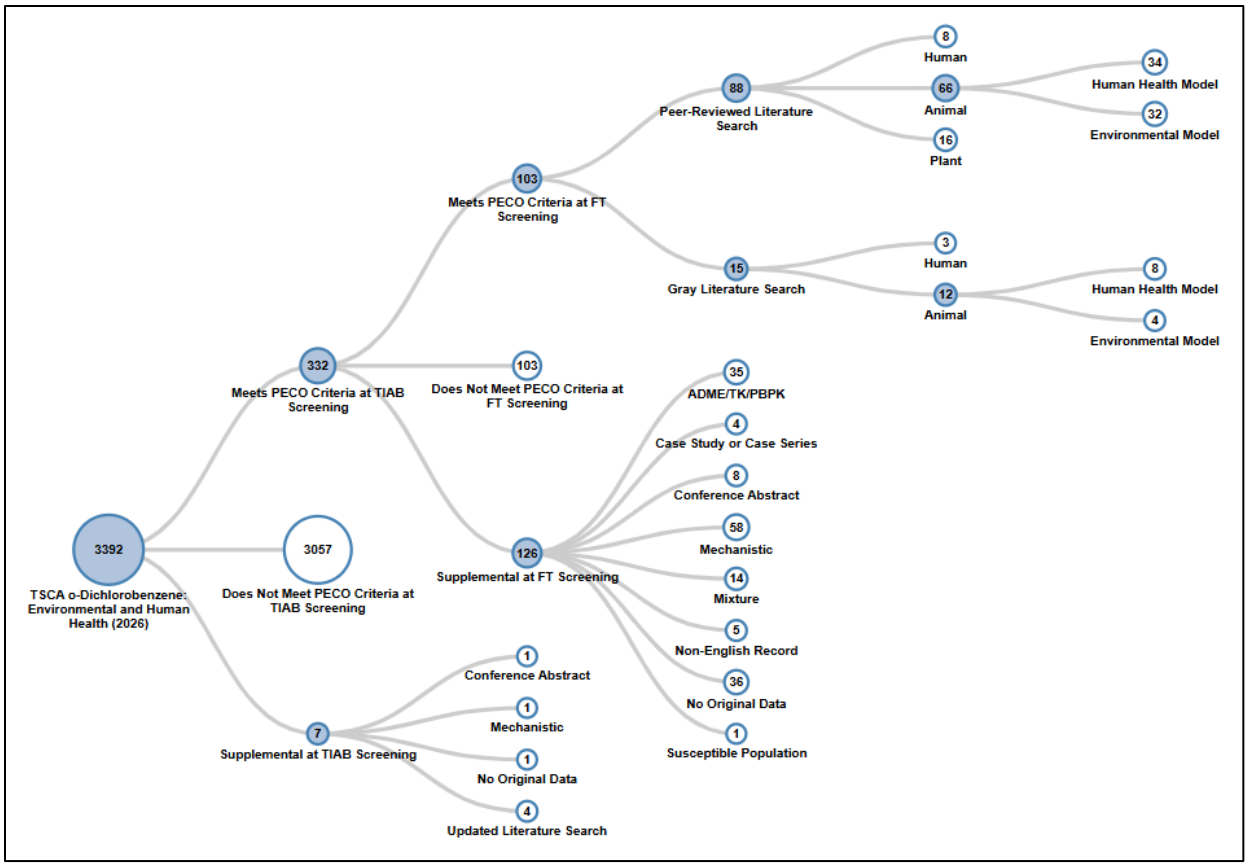
Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	6.96E00	5	
Daphnid	48h	LC50	4.49E00	5	
Green Algae	96h	EC50	5.66E00	6.4	
Fish		ChV	7.91E-01	8	
Daphnid		ChV	6.24E-01	8	
Green Algae		ChV	1.97E00	8	
Fish (SW)	96h	LC50	8.83E00	5	

Appendix J LITERATURE INVENTORY TREES

During data screening, EPA followed the process described in Appendix H, Section H.5.11 of the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021), to conduct title-abstract (TIAB) and full-text screening for *o*-dichlorobenzene literature search results, as guided by the PECO statement. PECO stands for Population, Exposure, Comparator or Scenario, and Outcomes for Exposure Concentration or Dose. Full details and screening results for all the identified studies will be described in the *Draft Systematic Review Protocol for o-Dichlorobenzene*, to be released with the risk evaluation package (U.S. EPA, 2026k).

J.1 Hazard

The same PECO statement was used during TIAB and full-text screening for references obtained during the initial 2019 search for the evaluation of environmental and human health hazard (including both epidemiology and animal toxicology) resulting from exposure to *o*-dichlorobenzene. An updated PECO was used for the April 2025 literature update that focused on *in vivo* information specific to cancer. Figure_Apx J-1 presents the number of references that report environmental and human health hazard data that met PECO screening criteria at TIAB and full-text screening for *o*-dichlorobenzene.



Figure_Apx J-1. Literature Inventory Tree for Environmental and Human Health Hazard
View the interactive literature inventory tree in HAWC. Data in this figure represent all references obtained from the publicly available databases and gray literature references that were included in systematic review as of February 11, 2026.

J.1.1 Further Filtering of Epidemiological Studies

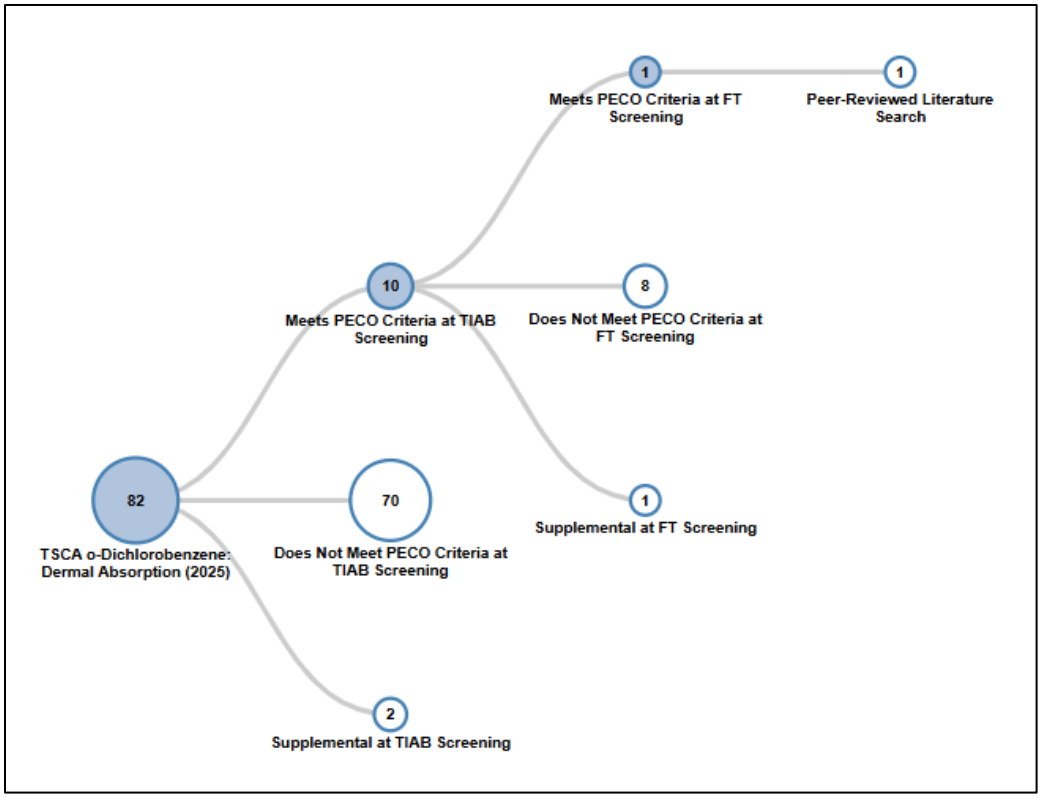
Table_Apx J-1 below presents the results of further filtering on epidemiological studies that passed PECO screening. Studies that included fewer than three exposure levels did not meet filtering criteria and did not undergo data quality evaluation or extraction. Studies with three or more exposure levels did undergo data quality evaluation (U.S. EPA, 2026f) and extraction (U.S. EPA, 2026d).

Table_Apx J-1. Further filtering results for epidemiological studies that passed PECO screening

Reference	Study Met PECO Criteria But Did Not Meet Further Filtering criteria (No Data Quality Rating)	Study that Met Both PECO Criteria and Further Filtering Criteria (Data Quality Evaluated)
(Kato et al., 2004)	Yes	
(Chrostek and Thoburn, 1976)	Yes	
(Rodrigues et al., 2020)		Yes
(Heck et al., 2015)		Yes
(Von Ehrenstein et al., 2016)		Yes

J.2 Dermal Absorption

EPA developed a PECO statement to conduct both TIAB and full-text screening of references considered for the evaluation of dermal absorption resulting from *o*-dichlorobenzene exposure. EPA additionally identified supplemental studies that may also inform dermal absorption and exposure for DINP. Each reference was manually screened by two reviewers at the TIAB and full-text screening steps or only at full-text, as relevant for the type of data source (peer vs. gray). Results are presented in Figure_Apx J-2.



Figure_Apx J-2. Literature Inventory Tree for Dermal Absorption

View the interactive literature inventory tree in HAWC. Data in this figure represent all references obtained from the publicly available databases and gray literature references that were included in systematic review as of December 12, 2025.

Appendix K BODY WEIGHT CHANGES

K.1 Human Evidence

Relevant human studies investigating body weight effects following exposure to *o*-dichlorobenzene have not been identified.

K.2 Laboratory Animal Evidence

Reduced body weight was observed in several animal studies covering various species and both sexes; however males were consistently more sensitive.

Oral

In the 10- and 90-day oral study in rats, decreased body weight gain of 10% to 15% was reported in males but not female rats following 10 days of 300 mg/kg-day exposure or 400 mg/kg-day for 90 days without any correlation with food consumption (Robinson et al., 1991).

In the 14-day range-finding experiment within the chronic oral bioassay, decreased body weight of up to 19% was also observed in rodents administered up to 500 mg/kg-day for 14 days (rats) or 13 weeks (mice and rats). Males were more sensitive in rats and females in mice; neither species demonstrated consistent body weight reduction over 2 years at approximately 100 mg/kg-day.

Inhalation

In the 1958 study described earlier, decreased body weight was reported in male but not female rats exposed to 93 ppm but not 49 ppm for 6 to 7 months, without any effects on guinea, pigs, rabbits, or mice exposed to the same concentrations (Hollingsworth et al., 1958).

In a two-generation inhalation study with rats, body weight was affected in both sexes and generations (Biodynamics, 1989). Body weight was significantly increased at 150 ppm and above in F0 males at most measured weeks as early as 1 week into the exposure period and at 50 ppm and above (all doses) at 20 or 21 to 24 weeks of exposure. F0 females also had reduced body weight in 3 of the 4 measurements between 20 and 24 weeks of exposure. Decreased weight was also observed at the 150 ppm and above level at earlier measurements of F1 animals, but reduced body weight in the F1 generation was most consistently observed in high-dose males. There was not any increased sensitivity in pregnant females. These reductions corresponded with increased food consumption, so reduced food does not explain the reductions in body weight.

Developmental and reproductive studies also demonstrated effects on body weight in parents as well as offspring post-weaning. In a developmental toxicity study on rats and rabbits, maternal body weight was reduced in pregnant rats exposed during gestation, with the absolute weight reduced at 200 ppm and above on GD 16 and at 400 ppm only on days 9 and 12 (Hayes et al., 1985). Body weight gain was significantly reduced at all concentrations (100 ppm and above) from GDs 6–8, 12–15, and overall, from GDs 6–20. Similar effects were observed in rabbits, with decreased maternal body weight at 100 ppm as measured on day 19 and 29 and reduced body weight gain at all doses from GDs 6–8, although there was an inconsistent dose-response

across concentrations. Food consumption was only reported as slightly decreased for the first 3 days of exposure in rats.

K.3 Mechanistic and Supporting Evidence

Body weight reduction indicates broad, systemic toxicity affecting multiple organ systems. It is therefore unlikely that any mechanism or MOA underlies the effects of *o*-dichlorobenzene on body weight. Based on the consistent effects across species, sexes, durations, and exposure routes there does not appear to be clear evidence of increased susceptibility in pregnant dams, and in some cases males were more sensitive than females.

K.4 Summary

As mentioned above, effects on body weight are unlikely to have a single, specific MOA. For *o*-dichlorobenzene, body weight changes are seen more consistently than many organ-level effects and occasionally at lower doses/concentrations. Body weight changes are therefore identified as a standalone, independent effect (as opposed to merely an indicator of MTD).

In considering the reasonably available information, EPA determines that adverse reductions in body weight are supported by robust animal data demonstrating reduced body weight following *o*-dichlorobenzene exposure for various durations, in multiple species, and via both oral and inhalation routes. The human and mechanistic data are indeterminate due to an absence of any studies. Based on the weight of scientific evidence, evidence integration judgements, and available dose-response data for adverse body weight changes, EPA considers the body weight change results to be appropriate for conducting dose-response assessments.

K.5 Dose-response analysis for reduced body weight

Decreased body weight was reported in several studies. Reduced body weight is typically only seen at higher doses indicating toxic overloading of biological systems, body weight effects occur at lower doses/concentrations of *o*-dichlorobenzene than many other hazard outcomes. The body weight changes from (Hayes et al., 1985) were the most sensitive, with decreased maternal body weight observed at the lowest dose of 100 ppm. While this could be considered a developmental effect, body weight reduction was consistently seen in non-gestational scenarios as well, although pregnancy could increase susceptibility to the health outcome. The body weight effects from (Hayes et al., 1985) were from less than two-weeks of exposures and were more sensitive than results from longer-term studies. Given the inverse dose-response for other effects (*i.e.,* shorter durations being more sensitive than longer), it is likely that effects on body weight follow a similar duration-response.

Inhalation

Reduced body weight or body weight gain was observed in pregnant female rabbits and rats from (Hayes et al., 1985). While this was a reproductive study, body weight reduction is seen consistently across sexes and in different study designs. Therefore, pregnant dams are considered a sensitive PESS group for this endpoint but the effect is not specifically a maternal effect, and the LOAEL for this study was lower than other body weight effects. EPA attempted to model multiple statistically significant endpoints from both rats and rabbits. The successful modeling results considered for POD selection are summarized below in Table_Apx K-1, all for rat endpoints. Absolute maternal body weight gain in rats were successfully modeled, however the

standard 10% RD BMR applied to body weight resulted in a BMDL greater than the highest dose which did not match the data; this result is not presented below. Instead, the BMDL based on the default 1 SD BMR for continuous datasets was applied. The largest magnitude of response was reduced maternal body weight gain in rats from GD 6–8; this could not be modeled however because of the negative body weight gain values. Therefore, the dataset was transformed by adding +10 to each value, and 1 control SD was used for the BMR. Reduced maternal body weight gain for the full measured period of GD 6–20 did successfully model with the standard BMR of 10% RD and default 1 control SD. Both values when running the default model sets (*i.e.,* not all restricted models) were higher than the LOAEC, however, discounting consideration of those results compared to the other options.

Table_Apx K-1. Dose-Response Analysis of Selected Endpoints for Deriving Systemic Toxicity PODs for Chronic Inhalation Exposure Scenarios

Reference and Study Details	Study POD/ Type (HEC)	Effect/Dataset Modeled	HEC/HED (Units)	UF[a]
Hayes et al. (1985) Female Fischer 344 rats and New Zealand White rabbits exposed during gestation (GD 6–15 in rats, 6–18 in rabbits); 0, 100, 200, 400 ppm; 6 h/day; developmental toxicity study Systemic toxicity/reduced body weight, inhalation	LOAEC = 100 (HEC = 25.0) ppm	Reduced absolute maternal body weight in rats	$BMDL_{1SD}$ = 49.0 (ppm)	UF_A = 3 UF_H = 10 *Total UF = 30*
		Reduced maternal body weight gain in rats from GD 6-8 (transformed)	$BMDL_{1SD}$ = 17.8 (ppm)	
		Reduced maternal body weight gain in rats from GD 6-20	$BMDL_{10}$ = 32.8 (ppm)	
[a] UF determination is discussed in Section 2.3.1.				

The most sensitive POD from (Hayes et al., 1985) was a $BMDL_{1SD}$ of 17.8 ppm from reduced maternal body weight gain in rats from GD 6–8. There is some uncertainty in this value because the adversity of reduced weight gain over only a few days is unclear, and a statistical transformation was required to model the dataset (which had negative values). When modeling the same endpoint over the full measured period of GD 6–20, which did not require any statistical adjustment, the resulting $BMDL_{10}$ resulted in a very similar value of 32.8 ppm. The more sensitive value of 17.8 ppm is within the range of intermediate liver endpoints (Section 2.3.2.4). The equivalent HED is 19.7 mg/kg-day.

Appendix L SCREENING LEVEL CHRONIC TOXICITY POINT OF DEPARTURE (POD) FOR CHRONIC ORAL/DERMAL EXPOSURES

The most sensitive outcome from the chronic segment of (NTP, 1985) was kidney toxicity indicated by increased renal tubular regeneration in female mice. Unlike kidney toxicity in male rats associated with α2μ-globulin which is not relevant to humans, kidney toxicity in mice is assumed to be applicable to humans. This endpoint was not detected at 13 weeks of exposure in the same study. In other studies, kidney weights were increased in female rats at 188 mg/kg-day and above following at least 6 months of exposure (Hollingsworth et al., 1958) and at 90 days of 400 mg/kg-day (Robinson et al., 1991) but not in pregnant female rats or rabbits exposed up to 400 ppm for less than two weeks (Hayes et al., 1985). Kidney toxicity thus appears to be the only health effect for *o*-dichlorobenzene more sensitive to longer duration exposures; based on rodent data, kidney toxicity likely requires long term exposures of years in humans. While most kidney effects occurred in male rats, increased kidney weights in female rats in other studies provide some supporting evidence. Therefore, this result was also selected for dose-response analysis as the most sensitive effect identified from a chronic study.

Kidney was a target organ in the five-day toxicology and transcriptomics study (U.S. EPA, 2026l; NIEHS, 2025a). Therefore, EPA also considered transcriptional PODs for kidney based on gene sets associated with the biological processes of apoptosis, cytotoxicity, inflammation, oxidative stress, and necrosis (Section 2.3.2.2). As previously stated above and in Section 2.3.1, kidney toxicity appears to require sustained, long-term exposure for the majority of a rodent's lifetime, so there is substantial uncertainty in interpreting the transcriptomic response from a five-day exposure despite the relevance of these biological processes to the apical effect. Additionally, there is insufficient mechanistic information underlying the cause of the kidney damage (observed as tubular regeneration in (NTP, 1985)) and there is statistical uncertainty in the mouse tPOD results due to a small sample size of both tissue samples and activated genes. Therefore, the kidney tPOD is considered supplementary; the analysis can be found in Appendix C.

L.1 Dose-Response Analysis for Kidney Toxicity

Kidney damage indicated by a dose-responsive increase in tubular regeneration in female mice was modeled from (NTP, 1985). The result was successfully modeled with a BMR of 10% extra risk.

Table_Apx L-1. Dose-Response Analysis of Selected Endpoints for Deriving Systemic Toxicity PODs for Chronic Inhalation Exposure Scenarios

Reference and Study Details	Study POD/ Type (HEC)	Effect/Dataset Modeled	HEC/HED (Units)	UF[a]
NTP (1985) Male and female F344/N rats and B6C3F$_1$ mice exposed for 2 years; 0, 30, 60, 125, 250, 500 mg/kg-day; 5 days/week; chronic toxicity/carcinogenicity study Liver toxicity, oral gavage	NOAEL = 60[b] (HED = 5.6) mg/kg-day	Incidence of tubular regeneration indicative of kidney damage in female mice	$BMDL_{10}$ = 4.0 (mg/kg-day)	UF_A = 3 UF_H = 10 *Total UF = 30*
[a] UF determination is discussed in Section 2.3.1.				

L.2 Weight of Scientific Evidence Evaluation for Screening Level Oral/Dermal Chronic POD

The HED based on a $BMDL_{10}$ of 4.0 mg/kg-day for kidney toxicity is the only POD from a chronic study. When converting to an inhalation HEC (3.6 ppm), it is several fold more sensitive than the (Hayes et al., 1985) POD. The HEC and HED for kidney toxicity will therefore be used for risk estimation of all other systemic toxicity effects following chronic exposure; this POD is not applicable to less-than-chronic exposures. There is much more limited support for this endpoint compared to respiratory toxicity and liver toxicity; this endpoint is therefore considered supplementary for screening.

Appendix M OTHER NON-CANCER HAZARD STUDYIES

M.1 Hollingsworth et al. (1958) Study

M.1.1 Epidemiological Data

Respiratory Effects

An older study (Hollingsworth et al., 1958) included Dow Chemical Company industrial hygiene surveys of all plant operations in which workers handled *o*-dichlorobenzene tasks. In Dow Chemical's survey, 40 air samples from the workroom showed concentrations ranging from 1 to 44 ppm, with an average of 15 ppm. All workers employed by this company who handled *o*-dichlorobenzene had medical examinations, and the survey did not report any health effects from *o*-dichlorobenzene inhalation exposure at an average of 15 ppm. In addition, Hollingsworth (1958) showed that toxicology researchers did not complain of eye or nasal irritation while performing animal toxicology experiments with an ambient concentration of 50 ppm (Hollingsworth et al., 1958). Hollingsworth (1958) and ATSDR (ATSDR, 2006) cited the reference (Elkins, 1950) to report ocular and respiratory irritation at air concentrations of 100 ppm in a workplace setting (ATSDR, 2006). Even though these studies did not compare health impacts between exposed and non-exposed workers, they showed human health effects across a range of *o*-dichlorobenzene exposure concentrations. Overall, these human studies provide only slight weight of scientific evidence.

Kidney Effects

Hollingsworth et al. (1958) showed that the Dow Chemical Company industrial hygiene survey reported all plant operations in which workers handled *o*-dichlorobenzene and collected 40 air samples from the workroom with *o*-dichlorobenzene concentrations ranging from 1 to 44 ppm, averaging 15 ppm. All workers employed by this company who handled *o*-dichlorobenzene underwent medical examinations, and none reported any health effects from inhalation exposure to *o*-dichlorobenzene at an average of 15 ppm. These human survey reports contribute little to the weight of scientific evidence due to a small air sample size and the lack of comparison of health impacts between exposed and unexposed groups.

M.1.2 Animal Data

Liver Effects

Exposure to an air concentration of 977 ppm for 1 hour (but not 30 min), 539 ppm for 3 hours, or 539 ppm for 6.5 hours caused increased liver weight and central lobular necrosis in rats (Hollingsworth et al., 1958). In the same study, exposure to 93 ppm via inhalation for 5 days/week for 6-7 months did not cause any adverse liver effects in rats, guinea pigs, rabbits, or monkeys, nor did exposure to 49 ppm for 6.5 months in rats, guinea pigs, or mice. The same study did observe increased liver weight in female rats (males not tested) following 5 days/week of oral exposure to 188 mg/kg-day and above for 6 months, with cloudy swelling seen at the 376 mg/kg-day dose level. Increased weight at 18.8 mg/kg-day was not statistically significant; however $p = 0.02$ was used as a cutoff instead of the $p = 0.05$ (Hollingsworth et al., 1958).

Respiratory Effects
The study from Hollingsworth et al. (1958) also included animal toxicology experiments; there were no effects on lung weight or histology in rats, guinea pigs, rabbits, or monkeys exposed for 5 days/week to air concentrations of 93 ppm for 6 to 7 months.

Kidney Effects
In the same older study from Hollingsworth describing human evidence above (1958), there were no effects on kidney weight, histology, or clinical biomarkers of damage in urine of rats, guinea pigs, rabbits, or monkeys exposed for 5 days/week to 93 ppm for 6 to 7 months. Effects were also not observed in guinea pigs, mice, or rats exposed to 49 ppm for the same duration. The same study did report dose-responsive increased kidney weight following 5 days/week oral exposure (tested on female rats only) to 18.8, 188, or 376 mg/kg-day for 6 months (significant at $p = 0.02$ or less at 188 and 376 mg/kg-day). Increased kidney weight was also observed in male rats (females not tested) following a single exposure for up to 1 hour to 977 ppm or up to 6.5 hours to 539 ppm, along with cloudy swelling of the kidney tubular epithelium.

Reproductive/Developmental Effects
Repeated dose systemic toxicity studies did not report any effects on male or female reproductive organs. In an older study testing different lengths of exposure on male rats and guinea pigs, there were no reported changes in testicular weight or histology via inhalation exposed to 93 ppm *o*-dichlorobenzene for 6 to 7 months (Hollingsworth et al., 1958).

Body Weight
In the 1958 study described earlier, decreased body weight was reported in male but not female rats exposed to 93 ppm but not 49 ppm for 6 to 7 months, without any effects on guinea, pigs, rabbits, or mice exposed to the same concentrations (Hollingsworth et al., 1958).

M.2 Dow Chemical Study (Dow Chemical, 1992)

Liver Health Effect. Dow Chemical Company (1992) published an occupational health summary report, which included industrial hygiene monitoring for air samples in 1989–1991 in an occupational setting and tested the liver enzymes as the liver function for seven contractor workers. Air monitoring detected seventeen TSCA 8(d) chemicals present. These workers were exposed to various chlorinated hydrocarbons, in addition to *o*-dichlorobenzene. The occupational health summary report showed that one of the seven individuals tested had a mild elevation of two of the five liver enzymes tested. The overall study results do not suggest any toxic effect on the liver. However, due to the limitations in multiple chemical exposure and a small sample size, this report ‘s results place low weight on the scientific evidence.

M.3 Other Limited Animal Toxicology Studies

Severe necrosis and “fatty changes” associated with porphyria in three male rats resulted from 15 days of exposure to 455 mg/kg-day via oral gavage (Rimington and Ziegler, 1963). In an acute oral screening study that used only one rat per dose in a series ranging from 6 mg/kg to 1784 mg/kg, two serum biochemical markers of liver damage, serum ALT and serum AST, were increased by at least 100% at the 172 mg/kg dose level or above (Allis et al., 1992).