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Draft Environmental Hazard Assessment for Diisobutyl Phthalate (DIBP)

Technical Support Document for the Draft Risk Evaluation

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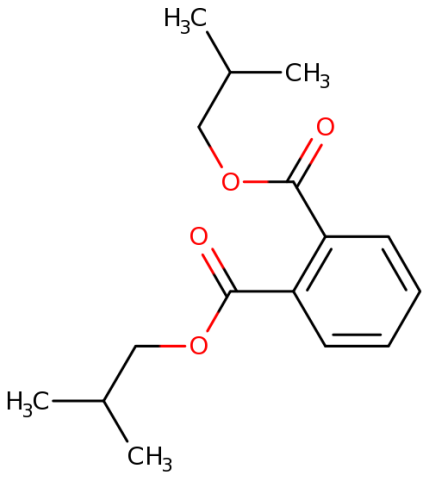

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ABBREVIATIONS AND ACRONYMS

AF	Assessment factor
ChV	Chronic value
COC	Concentration(s) of concern
EC50	Effect concentration at which 50 percent of test organisms exhibit an effect
EPA	Environmental Protection Agency
HC05	Hazard concentration that is protective of 95 percent of the species in the SSD
HV	Hazard value
LC50	Lethal concentration at which 50 percent of test organisms die
LD50	Lethal dose at which 50 percent of test organisms die
LOEC	Lowest observable effect concentration
LOAEL	Lowest observable adverse effect level
LOEC	Lowest observable effect concentration
LOEL	Lowest observable effect level
MATC	Maximum acceptable toxicant concentration
NAM	New approach methodology
NITE	National Institute of Technology and Evaluation
NOEC	No observable adverse effect concentration
NOAEL	No observable effect level
NOEC	No observable effect concentration
NOEL	No observable effect level
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPT	Office of Pollution Prevention and Toxics
POD	Point of departure
QSAR	Quantitative structure-activity relationship (model)
SSD	Species sensitivity distribution
TRV	Toxicity reference value
TSCA	Toxic Substances Control Act
U.S.	United States
Web-ICE	Web-based Interspecies Correlation Estimation

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SUMMARY

EPA considered all reasonably available information identified through its systematic review process under the Toxic Substances Control Act (TSCA) to characterize environmental hazard endpoints for DIBP. Upon evaluating the reasonably available information, environmental hazard thresholds were derived for aquatic vertebrates, aquatic invertebrates, aquatic benthic invertebrates, aquatic plants and algae, terrestrial vertebrates, terrestrial invertebrates, and terrestrial plants.

The acute aquatic concentration of concern (COC) for DIBP was derived from a species sensitivity distribution (SSD) that contained empirical 96-h LC50s for nine species identified in systematic review as well as an additional 72 species with predicted LC50 and EC50 values from the Web-Based Interspecies Correlation Estimation (Web-ICE) (v4.0) toxicity value estimation tool (Raimondo, 2010). The SSD was developed using the The SSD Toolbox (v1.1), which is a resource created by EPA's Office of Research and Development (ORD) that can fit SSDs to environmental hazard data (Etterson, 2020). To address data gaps in the DIBP environmental hazard data set, Dibutyl Phthalate (DBP) was used as an analog and read-across was conducted from the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a). Of the nine studies identified in systematic review and used in the SSD, two studies were from the DIBP empirical data set and seven were from the DBP empirical data set. The acute COC for aquatic vertebrates, invertebrates, and benthic invertebrates was identified as 287 µg/L. All chronic aquatic COCs were calculated using read-across from DBP as an analog. The chronic aquatic vertebrate COC was identified as 1.56 µg/L, the aquatic invertebrate COC was 12.23 µg/L, the aquatic benthic invertebrate COC was 114.3 mg/kg dry sediment, and the algae COC was 31.6 µg/L.

Wildlife mammalian hazard data were not reasonably available; therefore, ecologically relevant reproductive endpoints from laboratory rodent studies were used to derive a hazard value for terrestrial mammals. Empirical DIBP toxicity data for rats were used to estimate a hazard value for terrestrial mammals at 353 mg/kg-bw/day. The terrestrial invertebrate hazard threshold for DIBP was identified as 14 mg DBP/kg dry soil based on read-across from DBP and the terrestrial plant hazard threshold for DIBP was identified as 10 mg DBP/kg dry soil based on a read-across from DBP (U.S. EPA, 2024a). EPA's rationale for selecting these hazard thresholds is described in Section 6.

1 INTRODUCTION

This technical support document is in support of the *Draft Risk Evaluation for Diisobutyl Phthalate (DIBP)* (U.S. EPA, 2025b). Diisobutyl Phthalate (DIBP) is a common name for the chemical substance 1,2-Benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester (CASRN 84-69-5). See draft risk evaluation for a complete list of all the technical support documents for DIBP (U.S. EPA, 2025b). DIBP is an organic substance primarily used as a plasticizer in a wide variety of consumer, commercial and industrial products. DIBP may be released during industrial activities, manufacturing, disposal, and through consumer use, with most releases occurring into air and water (U.S. EPA, 2024b). EPA reviewed studies of the toxicity of DIBP and its analog DBP to aquatic and terrestrial organisms and DIBP's potential environmental hazards.

2 APPROACH AND METHODOLOGY

During scoping and problem formulation, EPA reviewed potential environmental hazards associated with DIBP. EPA identified sources of environmental hazard data shown in Figure 2-10 of the *Scope of the Risk Evaluation for DIBP* (U.S. EPA, 2020b). 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, 2021a) and *Draft Risk Evaluation for Diisobutyl Phthalate (DIBP) – Systematic Review Protocol* (U.S. EPA, 2024f). Studies were assigned overall quality determinations of high, medium, low, or uninformative. EPA systematically evaluated all data for this hazard characterization but relies upon only high-quality and medium-quality studies for purposes of risk characterization.

Due to limited environmental hazard data for DIBP, DBP was used as an analog to fill data gaps (U.S. EPA, 2024a). The criteria for selecting an appropriate analog are structural similarity, similar physical, chemical, environmental fate and transport behavior in water and sediment, and similar ecotoxicological behavior in aquatic and benthic taxa based on DIBP toxicity predictions generated using ECOSAR in comparison to analog (DBP) empirical hazard data. For more information on selecting an analog, see Appendix A.

An SSD analysis was conducted using EPA's SSD Toolbox (v1.1) to determine an acute aquatic hazard threshold. A SSD is a type of probability distribution of toxicity values from multiple species. It can be used to visualize which species are most sensitive to a toxic chemical exposure, and to predict a concentration of a toxic chemical that is hazardous to a percentage of test species. Predicted hazard data were generated using EPA's Web-ICE (v4.0) toxicity predictions tool (Raimondo, 2010). Empirical data that were included in the SSD analysis were limited to at or below the limit of water solubility of 6.2 mg/L for DIBP (U.S. EPA, 2024d). The specific species and corresponding empirical data are outlined in Section 3 and a description on the SSD as well as values predicted through EPA's Web-ICE tool can be found in Appendix B.

3 AQUATIC SPECIES HAZARD

EPA reviewed a total of three studies for DIBP toxicity to aquatic organisms and 171 studies for DBP. Of these studies, those that received an overall quality determination of low or uninformative were not considered for quantitative risk evaluation. Further, studies that received an overall quality determination of high and medium, but demonstrated no acute or chronic adverse effects at the highest concentration tested (unbounded no-observed-effect-concentration [NOECs]), or where hazard values exceeded the limit of solubility for DIBP in water as determined by EPA at 6.2 mg/L (U.S. EPA, 2024d), were excluded from consideration for development of hazard thresholds. Therefore, for DIBP, two studies were considered for the development of hazard thresholds as one aquatic algae study received an overall quality determination of low (described below). For all DBP studies that were excluded from the quantitative analysis, please see Appendix C of the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a). For the analog DBP, the hazard values from studies which were used to derive hazard thresholds were used as read-across for DIBP and are described below (Table 3-1). These hazard values were the most sensitive, had clear population-level fitness endpoints and were selected as the most appropriate in the DBP data set to represent hazard. For all data considered for the DBP risk evaluation see the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a).

Studies that received an overall quality determination of uninformative were not considered or included in the quantitative risk assessment. Additionally, studies that received an overall quality determination of low were supplemented with read across. EPA identified 21 aquatic toxicity studies (two DIBP studies and 19 DBP studies). The DIBP acute aquatic and benthic hazard data along with the acute DBP analog data described below were used to generate Web-ICE toxicity predictions for additional taxa representation. Specifically, predicted hazard data for 72 species were generated using EPA's Web-ICE tool, including predictions for 39 fish species, 31 invertebrate species, and 2 amphibian species (Table_Apx B-2)Empirical and predicted hazard values were used as input in an SSD analysis to determine an acute aquatic hazard threshold.

Toxicity in Aquatic Vertebrates

One acute aquatic vertebrate study was available for the quantitative assessment of potential hazards from DIBP exposure. An additional six studies with empirical acute aquatic DBP data were used as an analog for DIBP (Table 3-1). For DIBP acute aquatic vertebrates, EPA conducted a study in which fathead minnows were exposed to several phthalates, including DIBP and DBP, for 24 hours (Bencic et al., 2024) and a 24-hour mortality LC50 of 5.6 mg/L was identified for DIBP (Table 3-1). The additional six studies with analog DBP represent three species of aquatic vertebrates with six hazard values. In bluegill (*Lepomis macrochirus*), the 96-hour mortality LC50s for aquatic DBP exposure ranged from 0.48 to 1.2 mg/L (Adams et al., 1995; EG&G Bionomics, 1983b; Buccafusco et al., 1981). In rainbow trout (*Oncorhynchus mykiss*), the 96-hour mortality LC50s for aquatic DBP exposure ranged from 1.40 to 1.60 mg/L (EnviroSystem, 1991). In zebrafish (*Danio rerio*), a 72-hour mortality LC50 of 0.63 mg/L DBP was identified (Chen et al., 2014).

TSCA Section 4(h)(1)(B) requires EPA to encourage and facilitate the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions. In line with EPA's New Approach Methods Work Plan, EPA OPPT and ORD have been collaborating on developing new methods for use in TSCA risk evaluations. Specifically, a project was conducted to generate omics-based PODs and compared them to traditional endpoints using fathead minnow as the model organism for three of the phthalates undergoing a TSCA risk evaluation, including DIBP (Bencic et al., 2024). In this study, points of Departure (PODs) were derived for transcriptomic change (tPOD;

0.87 mg/L), metabolomic change (mPOD; 0.15 mg/L), and behavioral change (bPOD 0.90 mg/L) resulting from 24 hour duration of aquatic DIBP exposure to fathead minnows. These results suggest that fathead minnow larvae exhibited changes in gene expression, metabolite levels, and swimming behavior at sublethal concentrations of DIBP. While hazard thresholds are usually calculated with *in vivo* data measuring an apical endpoint (*e.g.*, mortality, reproduction, growth), these mechanistic (transcriptomic and metabolomic) and behavior points of departure represent potential information that may be used for reducing the time needed for toxicity testing in vivo and provide an alternate method to characterize hazard as well as provide important evidence for mechanisms of action. At this time, EPA has not used the omics-based PODs in the DIBP draft risk evaluation. There are uncertainties with respect to the extent to which these sub-organismal and individual-level effects (*e.g.*, behavior) at short exposure durations are comparable to ecologically relevant outcomes, such as survival and reproduction, in wild fish populations.

No chronic aquatic vertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, a read-across was conducted using the hazard value used to derive a hazard threshold identified from the DBP data set as an analog for chronic aquatic vertebrate hazard data. From the DBP hazard data set, 11 studies with overall quality determinations of high and medium contained chronic endpoints that identified definitive hazard values for five fish species and two amphibians (U.S. EPA, 2024d). The hazard threshold identified in DBP resulted from a multigenerational Japanese medaka (*Oryzias latipes*) study in which parental fish were aqueously exposed to DBP at measured concentrations of 15.6, 38.7, 66, 103, and 305 µg/L. Significant effects were observed in growth of both male and female F1 and F2 generations. In the male and female F1 generations, weight was significantly less compared to controls at 112-days, resulting in no observed effect concentrations/lowest observed effect concentration (NOECs/LOECs) of <15.6/15.6 µg/L and 66/103 µg/L DBP in males and females, respectively. Additionally, in the F2 generation, weight was significantly lower compared to controls at day 98, resulting in NOECs/LOECs of 103/305 µg/L and 15.6/38.7 µg/L DBP in males and females, respectively (EAG Laboratories, 2018). Unbounded effects (unbounded LOEC) were also observed for growth at the lowest concentration tested. Specifically, male F1 adult weight at 112-days, male F2 adult weight and length at 70-days, and male F2 adult length at 98-days were significantly inhibited at 0.015 mg/L DBP. The LOEC of 15.6 µg/L DBP for a reduced weight in the male F1 generation was chosen for COC calculations.

Toxicity in Aquatic Invertebrates

No acute or chronic aquatic invertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, a read-across was conducted using the acute and chronic hazard thresholds identified for aquatic invertebrates exposed to DBP. From the acute DBP hazard data set, four studies with hazard data for two aquatic invertebrate species were included in the SSD analysis for DIBP. In the opposum shrimp (*Americamysis bahia*), the mortality 96-hour LC50s ranged from 0.50 to 0.75 mg/L (Adams et al., 1995; EG&G Bionomics, 1984a). In the water flea (*Daphnia magna*), the 48-hour mortality LC50s ranged from 2.55 to 5.2 mg/L (Wei et al., 2018; McCarthy and Whitmore, 1985). From the DBP chronic hazard data set, eight studies contained endpoints that identified definitive hazard values for 10 aquatic invertebrate species. The hazard value chosen to derive a hazard threshold for chronic invertebrates resulted from a 14-day study of the Amphipod crustacean (*Monocorophium acherusicum*), which were maintained in measured aqueous concentrations of 0.044, 0.34, and 3.7 mg/L DBP. An observed 90 percent reduction in abundance was observed at 0.34 mg/L DBP resulting in 14-day NOEC/LOEC of 0.044/0.34 mg/L and a chronic value or geometric mean of the NOEC/LOEC (ChV) of 0.112 mg/L (Tagatz et al., 1983).

Toxicity in Aquatic Benthic Invertebrates

Acute invertebrate hazard data for DIBP was identified in one medium-rated study representing a 96-hour exposure to the harpacticoid copepod (*Nitocra spinipes*). The static 96-hour LC50 for mortality was measured at 3 mg/L DIBP (Linden et al., 1979) (Table 3-1). No additional acute and no chronic benthic invertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, a read-across was conducted using acute hazard studies for benthic invertebrates exposed to DBP as well as a read-across of the hazard value chosen to derive a hazard threshold from the DBP data set as an analog for chronic aquatic benthic invertebrate hazard. In the midge (*Paratanytarsus parthenogeneticus*) and the midge (*Chironomus plumosus*) DBP acute benthic invertebrate hazard data set, the 48-hour mortality LC50s ranged from 4.0 to 5.8 mg/L DBP (EG&G Bionomics, 1984b; Streufort, 1978). Acute aquatic hazard values were included in the SSD analysis.

From the DBP chronic benthic invertebrate hazard data set, the hazard threshold was identified from Call et al. (2001), which studied the effects of DBP in pore water and sediment for high, medium, and low TOC (total organic carbon) in the midge (*Chironomus tentans*). For high TOC, a 10-day NOEC/LOEC of 0.448/5.85 mg/L DBP in pore water and 508/3550 mg/kg dry weight DBP in sediment was observed for an increase in weight. For medium TOC, a 10-day NOEC/LOEC of 3.85/16 mg/L DBP in pore water and 423/3090 mg/kg dry weight DBP in sediment was observed for an increase in weight. For mortality, the 10-day NOEC/LOEC for pore water and sediment in high, medium, and low TOC was 0.448/5.85 mg/L DBP and 508/3550 mg/kg dry weight DBP, 3.85/16 mg/L DBP and 423/3090 mg/kg dry weight DBP, and 0.672/4.59 mg/L DBP and 50.1/315 mg/kg dry weight DBP, respectively (Call et al., 2001). The data resulting from the medium TOC sediment group was chosen to derive a COC as this is the closest to the assumed TOC level (four percent) used in Point Source Calculator (EPA, 2019) to estimate DIBP exposure in benthic organisms.

Toxicity in Amphibians

No amphibian studies were available for the quantitative assessment of potential hazards from DIBP exposure. Web-ICE predictions generated using both DIBP and DBP acute aquatic hazard data identified four 24-hour LC50s for two amphibian species. In the bullfrog (*Lithobates catesbeianus*), a 24-hour LC50 of 2.98 mg/L (a geometric mean of three predicted values for the same species) was predicted. In the African clawed frog (*Xenopus laevis*), a 24-hour LC50 of 4.0 mg/L was predicted. These data were used in the acute SSD analysis.

Toxicity in Aquatic Plants

One low-quality study was available for the assessment of potential hazards from DIBP exposure to aquatic algae. In this study, no effects were observed on population growth in algae (*Karenia brevis*) exposed to 0 to 200 ml/L DIBP for seven days (Liu et al., 2016). Since EPA relies upon only high-quality and medium-quality studies for purposes of quantitative risk characterization, a read-across was conducted using the hazard value chosen to derive a hazard threshold from the DBP data set as an analog for aquatic plant and algae hazard data. The DBP hazard data set contained three high- or medium-rated studies with endpoints that identified definitive hazard values for one species of algae (U.S. EPA, 2024d). The hazard value used to derive a hazard threshold for DBP resulted from a medium-quality green algae (*Selenastrum capricornutum*) study (Adachi et al., 2006), which identified a 96-hour NOEC/LOEC of 0.1/1.0 mg/L in *S. capricornutum* at DBP measured concentrations ranging from 0.1 to 10 mg/L (Adachi et al., 2006).

Table 3-1. Aquatic Organisms Environmental Hazard Studies Used for DIBP, Supplemented with

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	Test Organism	Hazard Values	Duration	Phthalate	Endpoint	Citation (Study Quality)
Aquatic Vertebrates						
Acute	Fathead minnow (*Pimephales promelas*)	5.3 mg/L[a]	24-hr LC50	DIBP	Mortality	(Bencic et al., 2024) (High)
Acute	Bluegill (*Lepomis macrochirus*)	1.2 mg/L[a]	96-hr LC50	DBP	Mortality	(Buccafusco et al., 1981) (Medium)
		0.85 mg/L[a]	96-hr LC50	DBP	Mortality	(EG&G Bionomics, 1983b) (High)
		0.48 mg/L[a]	96-hr LC50	DBP	Mortality	(Adams et al., 1995) (High)
	Rainbow trout (*Oncorynchus mykiss*)	1.60 mg/L[a]	96-hr LC50	DBP	Mortality	(EG&G Bionomics, 1983a) (High)
		1.40 mg/L[a]	96-hr LC50	DBP	Mortality	(EnviroSystem, 1991) (High)
	Zebrafish (*Danio rerio*)	0.63 mg/L	72-hr LC50	DBP	Mortality	(Chen et al., 2014) (Medium)
Chronic	Japanese medaka (*Oryzias latipes*)	<15.6/15.6 µg/L	112-d NOEC/ LOEC (ChV)	DBP	Growth – Weight male F1 Adults	(EAG Laboratories, 2018) (High)
Aquatic Invertebrates						
Acute	Opossum shrimp (*Americamysis bahia*)	0.75 mg/L[a]	96-hr LC50	DBP	Mortality	(EG&G Bionomics, 1984a) (High)
		0.50 mg/L[a]	96-hr LC50	DBP	Mortality	(Adams et al., 1995) (High)
	Water flea (*Daphnia magna*)	5.2 mg/L[a]	48-hr LC50	DBP	Mortality	(McCarthy and Whitmore, 1985) (Medium)
		2.55 mg/L[a]	48-hr LC50	DBP	Mortality	(Wei et al., 2018) (High)

	Test Organism	Hazard Values	Duration	Phthalate	Endpoint	Citation (Study Quality)
		4.31 mg/L[a]	48-hr LC50	DBP	Mortality	
		2.83 mg/L[a]	48-hr LC50	DBP	Mortality	
Chronic	Amphipod crustacean (*Monocorophium acherusicum*)	0.044/0.34 mg/L (0.122 mg/L)	14-d NOEC/ LOEC (ChV)	DBP	Population - Abundance	(Tagatz et al., 1983) (Medium)
Aquatic Benthic Invertebrates						
Acute	Harpacticoid copepod (*Nitocra spinipes*)	3 mg/L[a]	96-hr LC50	DIBP	Mortality	(Linden et al., 1979) (Medium)
	Harpacticoid copepod (*Nitocra spinipes*)	1.7 mg/L[a]	96-hr LC50	DBP	Mortality	(Linden et al., 1979) (Medium)
	Midge (*Paratanytarsus parthenogeneticus*)	5.8 mg/L[a]	48-hr LC50	DBP	Mortality	(EG&G Bionomics, 1984b) (High)
	Midge (*Chironomus plumosus*)	4.0 mg/L[a]	48-hr LC50	DBP	Mortality	(Streufort, 1978) (Medium)
Chronic	Midge (*Chironomus tentans*)	423/3090 mg/kg (1143 mg/kg) dry weight	10-d NOEC/ LOEC (ChV)	DBP	Mortality	(Call et al., 2001) (High)
Aquatic Plants and Algae						
	Green algae (*Selenastrum capricornutum*)	0.1/1 mg/L	96-h NOEC/ LOEC	DBP	Population (Abundance)	(Adachi et al., 2006) (Medium)

[a]Value used in SSD analysis and used to inform web-ice predictions. Water solubility of DBP = 11.2 mg/L and water solubility of DIBP = 6.2 mg/L.

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4 TERRESTRIAL SPECIES HAZARD

Two wildlife terrestrial studies were identified for DIBP, one with a quality determination of high and one with a quality determination of medium. These studies contained relevant toxicity data for the nematode (*Caenorhabditis elegans*) and the tobacco plant (*Nicotiana tabacum*). Additionally, in lieu of wild terrestrial mammal studies, two references for human health model organisms (Sprague-Dawley rats, *Rattus norvegicus*) were used to determine terrestrial vertebrate hazard values. These studies were used to determine the lowest and thus most conservative DIBP concentration that displayed apical endpoint effects (*e.g.*, survival, reproduction, growth) in rodents, and which could also serve as representative of hazard effects in wild mammal populations. These dietary DIBP concentrations were expressed as doses in mg/kg bw/day, and since body weight was normalized, EPA used this data as a screening surrogate for the effects on ecologically relevant wildlife species to evaluate chronic dietary exposure to DIBP. One high-quality study on the springtail (*Folsomia fimetaria*) and a high-quality study on bread wheat (*Triticum aestivum*) were also included to fill data gaps in the DIBP data set Terrestrial species hazard data are displayed in Table 4-1, as the most relevant for quantitative assessment.

Toxicity in Terrestrial Vertebrates

EPA reviewed two laboratory rodent studies from human health animal models for hazards of DIBP as surrogates to wild mammal populations, which contained ecologically relevant reproductive endpoints with both a no observed effect level (NOAEL) and lowest observed effect level (LOAEL) represented for each endpoint (Saillenfait et al., 2008; Saillenfait et al., 2006). EPA's decision to focus on ecologically relevant (population level) reproductive endpoints in the rat and mouse data set for DIBP for consideration of a hazard threshold in terrestrial mammals is due to the known sensitivity of these taxa to DIBP in eliciting phthalate syndrome (U.S. EPA, 2025a). EPA focused on studies which contained both a NOAEL and a LOAEL for each reproductive endpoint to refine the hazard threshold. Of the two rat studies containing NOAEL-LOAEL pairs for ecologically relevant reproductive endpoints, EPA selected the study with the most sensitive, and thus most conservative, LOAEL for deriving the hazard threshold for terrestrial mammals. In one study, pregnant Sprague-Dawley rats were given DIBP at doses of 0 (olive oil), 250, 500, 750, and 1000 mg/kg/day for 21 days via gavage. A significant decrease in maternal body weight gain was observed starting at gestational days six through nine at concentrations greater than 500 mg/kg/day and the percent of resorptions per litter was significant at 750 mg/kg/day (27.6 percent). In both male and female fetuses, body weight was significntly lower (9 percent) at 500 mg/kg/day compared to controls, resulting in a gestational day 20 NOAEC/LOAEC of 250/500 mg/kg/day (Saillenfait et al., 2006). This study was used for hazard value calculations. In the other study, pregnant Sprague-Dawley rats were given DIBP on gestation days 6-21 at doses of 0 (olive oil), 125, 250, 500, and 625 mg/kg/day via gavage. No effects were observed at any dose in pregnant females nor were any effects observed on litter size. However, at 500 and 625 mg/kg/day, male pup weight was lower than controls by six to eight percent and 10 to 12 percent, respectively. Additionally, male and female pup weight was significantly less than the control on post-natal day (PND) 1 at 625 mg/kg/day (Saillenfait et al., 2008).

Toxicity in Terrestrial Invertebrates

Acute terrestrial invertebrate hazard data for DIBP was identified in one high-ranking study. Nematodes maintained in culture media with DIBP for 24 hours at nominal concentrations of 0, 100, and 1000 mg/L DIBP were observed to have significant effects on behavior at 100 mg/L. Specifically, nematodes exhibited changes in distance moved, reversals, and overall movements at the lowest concentrations tested compared to controls (Tseng et al., 2013). However, this study only tested concentrations that exceeded the DIBP limit of water solubility (6.2 mg/L), therefore a read-across was conducted from the DBP data set. The DBP hazard data set contained 12 high- or medium-rated studies with definitive

endpoints that identified hazard values for seven terrestrial invertebrate species (U.S. EPA, 2024d). The hazard value used to derive a hazard threshold for DBP from was from a high-ranking study that examined the effects of DBP in the springtail (*Folsomia fimetaria*). In this study, adult springtail reproduction was significantly affected with an observed 21-day EC10 and EC50 of 14 and 68 mg/kg dry soil, respectively (Jensen et al., 2001).

Toxicity in Terrestrial Plants

One medium-ranking study was available to assess DIBP toxicity to terrestrial plants. The toxicity of DIBP to two tobacco plant (*Nicotiana tabacum*) seed cultivars, G168 and Hong da, was assessed using filter paper at nominal concentrations of 0.1, 0.5, 1.0, and 10 mM (0, 27.8, 139, 278, and 2,783 mg/L) DIBP. In the G168 cultivar, seed germination was significantly reduced (28 percent germination) at the highest concentration tested and in the Hong da cultivars, seed germination was significantly reduced (44 percent germination) at 0.5 mM. Thus, the 7-day NOEL/LOEL for seed germination was found to be 1.0/10 mM (278/2,783 mg/L) for G168 cultivars, and 0.1/0.5 mM (27.8/139 mg/L) for Hong da cultivars (Jia et al., 2011). However, this study only tested concentrations that exceeded the DIBP limit of water solubility (6.2 mg/L), therefore this data was not used in the quantitative assessment of DIBP hazards and read across was conducted from DBP for terrestrial plants. In bread wheat exposed to DBP at concentrations of 0, 5, 10, 20, 30, and 40 mg/L, a 40-day LOEL of 10 mg/kg DBP (lowest concentration used in the study) for reduced weight in bread wheat was observed (Gao et al., 2019).

Table 4-1. Terrestrial Organisms Environmental Hazard Studies Used for DIBP

Test Organism	Hazard Values	Duration	Phthalate	Endpoint	Citation (Study Quality)
Terrestrial Vertebrates					
Sprague-Dawley rat	250/500 mg/kg/day[a]	Gestational day 20 NOAEL/ LOAEL	DIBP	Reproduction	(Saillenfait et al., 2006) (High)
Sprague-Dawley rat	250/500 mg/kg/day	Gestational day 21 NOAEL/ LOEL	DIBP	Reproduction	(Saillenfait et al., 2008) (High)
Terrestrial Invertebrates					
Nematode (*Caenorhabditis elegans*)	<100/100 mg/L (culture media)[b]	24-hr NOEL/ LOEL	DIBP	Behavior	(Tseng et al., 2013) (High)
Springtail (*Folsomia fimetaria*)	14 mg/kg dry soil[a]	21-d EC10	DBP	Reproduction	(Jensen et al., 2001) (High)
Terrestrial Plants					
Tobacco (*Nicotiana tabacum*) G168 cultivar	278/2,283 mg/L[b]	7-d NOEL/ LOEl	DIBP	Reproduction - germination	(Jia et al., 2011) (Medium)
Tobacco (*Nicotiana tabacum*) Hong da cultivars	27.8/139 mg/L[b]		DIBP		
Bread wheat (*Triticum*	<10 mg/kg dry soil/10	40-day LOEL	DBP	Growth	(Gao et al., 2019) (High)

Test Organism	Hazard Values	Duration	Phthalate	Endpoint	Citation (Study Quality)
aestivum)	mg/kg dry soil[a]				
[a]Value used to derive a hazard value; [b]Value exceeds the DIBP limit of water solubility (6.2 mg/L)					

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5 WEIGHT OF SCIENTIFIC EVIDENCE CONCLUSIONS FOR ENVIRONMENTAL HAZARD ASSESSMENT

EPA determined that DIBP poses potential hazard to acute aquatic species at aquatic concentrations of 287 µg/L, as determined through SSD supplemented with DIBP empirical data, DBP empirical data, and predicted values calculated through Web-ICE. EPA determined that DIBP poses potential chronic hazard effects to aquatic species based on read-across conducted from DBP) (U.S. EPA, 2024a), which evaluated studies on DBP chronic toxicity in aquatic vertebrates, invertebrates, and benthic invertebrates as an analog to DIBP. The endpoints used in the read-across were the hazard values used to derive hazard thresholds in DBP, which were the most sensitive, clear population-level fitness endpoints selected as the most appropriate in the DBP data set to represent hazard. For all studies considered in the DBP hazard assessment, see the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a).

EPA determined that DIBP poses potential hazards to terrestrial mammals at a dietary dose of 353 mg/kg/day, which is supported by evidence taken from laboratory rodent studies used as human health models (Saillenfait et al., 2006). EPA determined DIBP poses potential hazards to terrestrial invertebrates based on read-across from DBP (U.S. EPA, 2024a), in which a hazard value of 14 mg/kg dry soil was identified (Jensen et al., 2001). EPA determined DIBP poses potential hazards to terrestrial plants based on read-across from DBP (U.S. EPA, 2024a), in which a hazard value of 10 mg/kg dry soil was identified (Gao et al., 2019).

The aquatic COCs and terrestrial hazard thresholds identified in this technical support document will be used in the *Draft Risk Evaluation for Diisobutyl Phthalate (DIBP)* (U.S. EPA, 2025b) to characterize environmental risk.

5.1 Strengths, Limitations, Assumptions, and Key Sources of Uncertainty for the Environmental Hazard Assessment

EPA has robust confidence that DIBP poses potential hazard to acute aquatic species at 287 µg/L. This data is supported through SSD analysis conducted with empirical data from two acute DIBP aquatic hazard studies, seven acute DBP aquatic hazard values, and supplemented with predicted values calculated through Web-ICE. A limitation and source of uncertainty in the assessment of hazards to chronic aquatic organisms is the lack of available data. No aquatic chronic studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, a read-across was conducted from DBP (U.S. EPA, 2024a). DBP was considered an appropriate analog for DIBP based on structural similarity, similar physical, chemical, environmental fate and transport behavior in water and sediment, as well as similar ecotoxicological behavior in aquatic taxa (Appendix A). EPA has robust confidence that DIBP poses hazard to aquatic vertebrates, invertebrates, and benthic invertebrates on a chronic basis. This robust confidence is supported by the quality and consistency of the analog DBP chronic aquatic vertebrate, invertebrate, and benthic invertebrate database. A read-across from DBP was also conducted for aquatic plants and algae. However, only one species of algae was available for the assessment of potential hazards from DBP (U.S. EPA, 2024a), therefore EPA has overall moderate confidence in the hazard for the DIBP aquatic plants and algae assessment. For more information on analog selection, see Appendix A.

In the terrestrial environment, EPA has moderate confidence that DIBP poses potential hazard to mammals, and robust confidence that DIBP poses potential hazard to invertebrates, and plants. The conclusion that DIBP poses hazard to terrestrial mammals at a dietary dose of 353 mg/kg/day, is supported by evidence obtained from laboratory rodent studies used as human health models. Utilizing

human health rodent models as a surrogate for terrestrial models introduces uncertainty into the terrestrial hazard characterization since these species may not fully represent effects observed in wild animal populations. The conclusion that DIBP poses hazard to terrestrial invertebrates is based on one study that identified significant behavioral effects in the nematode (Tseng et al., 2013). A limitation and uncertainty of the terrestrial invertebrate data set is the low number of available studies and species available to be used in the assessment. However, the strength of the database and identified hazard value is supported by the robust consistency, strength and precision, and biological gradient of the study results. EPA has moderate confidence that DIBP poses hazard to terrestrial plants. This confidence is supported by the quality and consistency of the analog DBP terrestrial plant database. Due to the added uncertainty from some studies in similar plants showing a lack of strong biologically relevant effects or clear dose-response, confidence was reduced for the strength and precision and dose-response considerations for the terrestrial plants assessment.

5.1.1 Confidence in the Environmental Hazard Data set

Based on the weight of the 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 evidence considerations and criteria detailed within the *Draft Systematic Review Protocol* (U.S. EPA, 2021a) guide the application of strength-of-evidence judgments for environmental hazard effect within a given evidence stream. See Appendix C for more information on the weight of scientific evidence conclusions and see
Table 5-1 for the confidence table that summarizes the information below.

For the acute aquatic assessment of DIBP, the database consisted of two studies, one with an overall quality determination of medium and another conducted by EPA with an overall quality determination of high. These two studies, plus data from seven additional studies from the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a), as well as 72 hazard endpoints obtained from Web-ICE predictions were used to generate an SSD output. Thus, for the acute data set, a robust confidence was assigned to the quality of the database. The studies from the analog DBP data set displayed similar effects on the same species across multiple studies and these effects were similar to what was observed in the two acute DIBP studies. Due to the observed consistent effects, a robust confidence was assigned to the consistency consideration for the acute aquatic assessment. The effects observed in both the acute DBP and DIBP data set were apical endpoints such as 48-hour, 72-hour, or 96-hour LC50s with additional predicted LC50 values reported from Web-ICE. Therefore, a robust confidence was assigned to the strength and precision consideration. As dose-response is a prerequisite of obtaining reliable LC50 values and was observed in the empirical studies that were used in the SSD, a robust confidence was assigned to the dose-response consideration. Lastly, for the acute aquatic assessment, mortality was observed in the empirical data for four fish and five invertebrates and mortality was predicted in 72 additional species using Web-ICE. The use of the lower 95 percent confidence interval (CI) of the 5th percentile hazardous concentration (HC05) in the SSD instead of a fixed assessment factor (AF) also increases confidence since it is a more data-driven way of accounting for uncertainty. Due to the use of empirical data combined with predicted data through a probabilistic approach, a robust confidence was assigned to the relevance consideration for the acute aquatic assessment.

No studies were available for the chronic aquatic vertebrate assessment of DIBP. Therefore, a read-across was conducted from DBP (U.S. EPA, 2024a). Eleven studies from the analog DBP contained chronic endpoints that identified definitive hazard values below the DIBP limit of water solubility for aquatic vertebrates (6.2 mg/L), (U.S. EPA, 2024d) for five fish species and two amphibians, resulting in robust confidence for quality of the database. DBP displayed chronic effects on growth which spanned

several orders of magnitude among aquatic vertebrate taxa, therefore a moderate confidence was assigned to the consistency of the database. In the study chosen to derive the COC, (EAG Laboratories, 2018), bodyweight in Japanese medaka was inhibited by 13.4 percent relative to the vehicle control, and there was a statistically significant trend toward greater body weight inhibition with increasing dose, culminating at 34.0 percent inhibition at the highest dose (305 µg/L). Strong dose-response effects were also observed in other studies in the DBP database. Therefore, a robust confidence was assigned to the strength and precision consideration and the dose-response consideration for the chronic aquatic invertebrate assessment. Lastly, due to ecologically relevant population level effects (growth and mortality) observed in multiple species for DBP, yet the data being represented by an analog, a moderate confidence was assigned to the relevance consideration for the chronic aquatic vertebrate assessment. All studies considered for DBP can be found in the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a).

No studies were available for the chronic aquatic invertebrate assessment of DIBP. Therefore, a read-across was conducted from DBP (U.S. EPA, 2024a). Eight studies from the analog DBP contained chronic endpoints that identified definitive hazard values below the DIBP limit of water solubility for 10 aquatic invertebrate species, resulting in robust confidence for quality of the database. The studies from DBP database had similar effects on the same species across multiple studies, and within one order of magnitude. Therefore, a robust confidence was assigned to the consistency consideration. In the study chosen to derive the COC (Tagatz et al., 1983), amphipod populations were reduced by 91 percent at the LOEC and 100 percent mortality was observed at higher doses. A strong dose-response relationship was also observed in the other studies from the analog DBP database and therefore a robust confidence was assigned to strength and precision and dose-response of the database for the chronic aquatic invertebrate assessment. For the chronic aquatic invertebrate assessment, ecologically relevant population level effects (mortality and reproduction) were observed in 10 species, two of which (water flea, *Daphnia magna*; and the worm *Lumbriculus variegatus*) are considered representative test species for aquatic toxicity tests. Similarly to the chronic aquatic vertebrate assessment, ecologically relevant population level effects were observed in multiple species for DBP, yet the data was represented by an analog, therefore a moderate confidence was assigned to the relevance consideration for the chronic aquatic invertebrate assessment. All studies considered for DBP can be found in the *Draft Environmental Hazard Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024a).

No studies were available for the chronic aquatic benthic invertebrate assessment of DIBP. Therefore, a read-across was conducted from DBP (U.S. EPA, 2024a). Three studies from the analog DBP contained chronic endpoints that identified definitive hazard values below the DIBP limit of water solubility for benthic invertebrates (U.S. EPA, 2024d). These studies included multiple species, endpoints, and durations, however only two species were represented. Additionally, the results seemed to be repeated across some of the studies and it was unclear in some cases whether the data were original. These considerations resulted in a slight confidence assigned for the quality of the database consideration. DBP studies were conducted with low, medium, and high TOC sediments. Among the same species, effects were generally within one order of magnitude in the same TOC. Therefore, a robust confidence was assigned to the consistency of the database. In the study chosen to derive the COC (Lake Superior Research Institute, 1997), the midge population was reduced by 76.7 percent at the LOEC (3,090 mg DBP/kg dry sediment) and population reduction in other treatments and TOC levels was consistent. Therefore, a robust confidence was assigned to the strength and precision of the database. In the medium TOC group, higher doses of DBP displayed similar mortality. Due to a clear dose-response relationship in other studies in the database, a moderate confidence was assigned to the dose-response consideration for the chronic benthic invertebrate assessment. Ecologically relevant population level effects were observed in two different species from the DBP database (scud, *Hyalella azteca*; and midge,

Chironomus plumosus), both of which are considered representative test species for benthic toxicity tests. However, relevance is limited by the use of an analog, therefore, moderate confidence was assigned to the relevance consideration for the chronic benthic invertebrate assessment.

No studies were available for the aquatic plant or algae assessment of DIBP. Therefore, a read-across was conducted from DBP (U.S. EPA, 2024a). DBP database consisted of seven high or medium quality studies for toxicity in aquatic plants and algae. Three studies from the analog DBP contained endpoints that identified definitive hazard values below the DIBP limit of water solubility (U.S. EPA, 2024d) for one species of green algae. Confidence in the database was reduced because only one species was identified and several of the studies in the database were not acceptable due to exposure concentrations being above the limit of solubility for DIBP, therefore a slight confidence was assigned for the quality of the database. DBP had similar effects on population, measured as either chlorophyll α concentration or cell abundance, in three independent studies. Thus, a robust confidence was assigned to the consistency of the database. In the study chosen to derive the COC (Adachi et al., 2006), a significant reduction in the algal population was observed at the LOEC (1000 µg/L DBP) and population reduction was increased with higher concentrations of DBP. However, there was an increase in algal population at the NOEC (100 µg/L DBP), therefore a moderate confidence was assigned to the strength and precision and dose-response considerations for the aquatic plants and algae assessment. An ecologically relevant population level effect (population abundance, measured as either chlorophyll α concentration or cell count) was observed in one species of green algae (*Selenastrum capricornutum*). Due to this species being considered a representative test species for algal toxicity tests, yet being the only species represented in the database and the use of an analog, moderate confidence was assigned to the relevance consideration for the aquatic plant and algae assessment.

For the terrestrial vertebrate assessment, EPA reviewed two laboratory rodent studies as surrogates from human health animal models for hazards of DIBP to wild mammal populations (Saillenfait et al., 2008; Saillenfait et al., 2006). While two terrestrial vertebrate studies were available for the assessment of DIBP, these studies were not from wildlife species and therefore a moderate confidence was assigned to the quality of the database. In these studies, effects on growth and reproduction were observed at NOAEL/LOAELs ranging from 250/500 mg/kg/day from to 500/750 mg/kg/day DIBP (Saillenfait et al., 2008; Saillenfait et al., 2006). Since significant effects occurred at similar doses and concentrations across studies, a robust confidence was assigned to the consistency of the database. In the study chosen to derive a terrestrial vertebrate hazard value, a significant reduction (seven percent) in body weight for both male and female fetuses resulting in a gestational day 20 NOEC/LOEC of 250/500 mg/kg/day was observed (Saillenfait et al., 2006). Body weight was also significantly reduced at the higher concentrations of 750 and 1000 mg/kg/day by 17 percent and 24 percent, respectively. Similar dose-response relationships were also observed for the other endpoints in the study. Thus, a robust confidence was assigned for the dose-response and strength and precision of the database considerations. Data from human-relevant terrestrial vertebrates (rat) were used to supplement the data set. A relevant population level effect (reproduction) was observed in this species. Yet because the study used to develop the hazard value was conducted in rats, which are less ecologically relevant than wildlife vertebrate species, a moderate confidence was assigned to the relevance consideration for the terrestrial vertebrate assessment.

No studies were reasonably available for the terrestrial invertebrate assessment of DIBP. Therefore, a read-across was conducted from DBP (U.S. EPA, 2024a). Two studies from the analog DBP contained endpoints that identified definitive hazard values below the DIBP limit of water solubility for two soil invertebrate species. A moderate confidence was assigned to the quality of the database because two terrestrial invertebrate species were represented by one high and one medium-rated study. In these two

species, the springtail (*Folsomia fimetaria*) and earthworm (*Eisenia fetida*), multiple endpoints were identified. While no inconsistencies were observed in the data, the most sensitive endpoint that was used to derive a hazard value was a 21-day EC10 in the springtail and since no other studies contained comparable endpoints, a moderate confidence evaluation was assigned to the consistency criterion. Due to a clear dose-response relationship and a strong biologically relevant effect in the DBP data set for soil invertebrates, a robust confidence was assigned to the strength and precision and dose-response criteria for the soil invertebrate assessment.

No studies were available for the terrestrial plant assessment of DIBP. Therefore, a read-across was conducted using DBP (U.S. EPA, 2024a). Most of the studies in the DBP database characterized doses in a way that was not useful for developing a hazard value (*e.g.*, in mg/m^3 soil fumigation). Therefore, slight confidence was assigned to the quality of the database. Since consistent growth effects were seen in a variety of species, but the observed effects were distributed over a wide range of concentrations, a moderate confidence was assigned to the consistency consideration. A dose-response effect was observed in the study used to derive a hazard threshold, but a clear dose response was not observed in all studies. Due to the added uncertainty from some studies in similar plants showing a lack of strong biologically relevant effects or clear dose-response, moderate confidence was assigned to the strength and precision and dose-response considerations for the terrestrial plants assessment.

Table 5-1. DIBP 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 (SSD)	+++	+++	+++	+++	+++	Robust
Chronic Aquatic Vertebrates	+++	++	+++	+++	++	Robust
Chronic Aquatic Invertebrates	+++	+++	+++	+++	++	Robust
Chronic Benthic Invertebrates	+	+++	+++	++	++	Moderate
Aquatic Plants & Algae	+	+++	++	++	++	Moderate
Terrestrial						
Terrestrial Vertebrates	+++	++	+++	+++	++	Moderate
Terrestrial Invertebrates	++	++	+++	+++	++	Robust
Terrestrial Plants	+	++	++	++	++	Moderate

[a] Relevance includes biological, physical/chemical, and environmental relevance.
+++ Robust confidence suggests thorough understanding of the scientific evidence and uncertainties. The supporting weight of the 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 the 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 the 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.

6 ENVIRONMENTAL HAZARD THRESHOLDS

EPA calculated hazard thresholds to identify potential concerns to aquatic and terrestrial species. After weighing the scientific evidence, EPA selected the appropriate toxicity value from the integrated data to use for hazard thresholds. Table 6-1 summarizes the aquatic concentrations of concern and Table 6-2 summarizes the terrestrial hazard values identified for DIBP. See Appendix C for more details about how EPA weighed the scientific evidence.

Aquatic Organism Threshold

For aquatic species, EPA uses probabilistic approaches (*e.g.,* SSD) when enough data are available (eight or more species) and deterministic approaches (*e.g.,* deriving a geometric mean of several comparable values) when limited data are available. A SSD is a type of probability distribution of toxicity values from multiple species. It can be used to visualize which species are most sensitive to a toxic chemical exposure, and to predict a concentration of a toxic chemical that is hazardous to a percentage of test species. This hazardous concentration is represented as an HCp, where p is the percent of species below the threshold. EPA used an HC05 (a Hazardous Concentration threshold for 5 percent of species) to estimate a concentration that would protect 95 percent of species. This HC05 can then be used to derive a COC, and the lower bound of the 95 percent CI of the HC05 can be used to account for uncertainty instead of dividing by an AF. For chronic exposures, an AF of 10 is used to account for uncertainty associated with increased exposure duration. EPA has more confidence in the probabilistic because an HC05 is representative of a larger portion of species in the environment. For the deterministic approach, a COC is calculated by dividing a hazard value by an AF according to EPA methods (U.S. EPA, 2016b, 2013, 2012).

Equation 6-1

$$COC = toxicity\ value \div AF$$

Terrestrial Organism Threshold

For terrestrial species, EPA estimates hazard by calculating a toxicity reference value (TRV), in the case of terrestrial mammals and birds, or by assigning the hazard value as the hazard threshold in the case of terrestrial plants and soil invertebrates. The TRVs generated for EPA's ecological soil screening levels (Eco-SSLs) are defined as doses, *"above which ecologically relevant effects might occur to wildlife species following chronic dietary exposure and below which it is reasonably expected that such effects will not occur"* (U.S. EPA, 2007, 2005a). EPA prefers to derive the TRV by calculating the geometric mean of the NOAELs across sensitive endpoints (growth and reproduction) rather than using a single endpoint. The TRV method is preferred because the geometric mean of NOAELs across studies, species, and endpoints provides greater representation of environmental hazard to terrestrial mammals and/or birds. However, when the criteria for using the geometric mean of the NOAELs as the TRV are not met, the TRVs for terrestrial mammals and birds are derived using a single endpoint. Due to a lack of available terrestrial data for DIBP, EPA used a deterministic approach and assigned a hazard value based on the most sensitive endpoint for each taxa.

6.1 Aquatic Species COCs

EPA derived three acute aquatic COCs and three chronic COCs using a combination of probabilistic and deterministic approaches with DIBP hazard data supplemented with a read-across from DBP. Plant and algae data was assessed separately and not incorporated into acute or chronic COCs because durations normally considered acute for other species (*e.g.,* up to 96 hours) can encompass several generations of algae. Section 3 summarizes the aquatic hazard thresholds.

Acute Aquatic Organism Threshold

The aquatic acute COC for DIBP was derived from an SSD that contained 96-h LC50s for nine species identified in systematic review (two species with DIBP hazard data and seven species with DBP hazard data), bolstered by an additional 72 predicted LC50 values from the Web-ICE toxicity value estimation tool. All studies included in the SSD were rated high or medium quality. After reviewing the possible statistical distributions for the SSD, the Metropolis Hastings 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, and evaluation of the line of best fit near the low-end of the SSD. The HC05 for this distribution is 406 µg/L. After taking the lower 95th percentile of this HC05 as an alternative to the use of assessment factors, the acute aquatic COC for vertebrates and invertebrates is **287 µg/L**. See Appendix B for details of the SSD that was used to derive the acute aquatic COC for DIBP. The SSD-derived acute aquatic COC is similar to the multiomics-based PODs derived by EPA (Bencic et al., 2024). Specifically, the PODs derived by EPA ranged from 150 µg /L (mPOD) to 900 µg /L (bPOD) (Tble_Apx D-1).

Chronic Aquatic Vertebrate Threshold

No chronic aquatic vertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, analog data on chronic aquatic vertebrate hazards from DBP exposure were used in a read-across to DIBP. The hazard value chosen to derive a hazard threshold resulted from a high-quality rated study on the Japanese medaka (*Oryzias latipes*) (EAG Laboratories, 2018). In this multi-generational study, the growth of F1 and F2 generations were significantly affected by exposure to DBP. Specifically, there was significant inhibition of body weight at the lowest concentration studied in the male F1 generation, with an unbounded LOEC value of 15.6 µg/L DBP. In the female F1 generation, the ChV for bodyweight inhibition was 0.082 mg/L DBP. In the F2 generation, the ChV for bodyweight inhibition in male fish was 0.117 mg/L DBP and 24.6 µg/L DBP in females. The most sensitive endpoints in this data set were for inhibition of bodyweight in F1 males (0.0015 mg/L) and F2 females (0.0246 mg/L). However, there was not a clear dose-response relationship for the body weight inhibition response as some of the higher concentrations of DBP displayed a smaller mean effected compared to the lower doses. Thus, this endpoint was not considered for the derivation of a COC. The most sensitive endpoint for which there was a reliable dose-response relationship between DBP exposure and reduced body weight was in F1 male fish, with a 112-day unbounded LOEC of 15.6 µg/L DBP. At this concentration, body weight was inhibited by 13.4 percent compared to the control and there was a clear dose-response relationship up to the highest concentration tested of 304 µg/L in which there was a 34 percent inhibition of body weight. Therefore, the hazard value was found to be 15.6 µg/L and after dividing by an AF of 10, the chronic aquatic vertebrate threshold is **1.56 µg/L**.

Chronic Aquatic Invertebrate Threshold

No chronic aquatic invertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, analog data on chronic aquatic invertebrate hazards from DBP exposure were used in a read-across to DIBP. The most sensitive hazard value resulted from a medium-quality rated study on the marine amphipod crustacean (*Monocorophium acherusicum*), which identified a 14-day ChV of 0.122 mg/L DBP for reduced population abundance (Tagatz et al., 1983). In this study, crustacean abundance was reduced by 91 percent at 0.340 mg/L resulting in a NOEC/LOEC of 0.044/0.340 mg/L DBP. The 14-day ChV for reduction in population abundance in the marine amphipod crustacean was selected to derive the chronic COC for aquatic invertebrates. After applying an AF of 10, the chronic COC for aquatic invertebrates is **12.23 µg/L**.

Acute Aquatic Benthic Invertebrate Threshold

The acute aquatic COC (287 µg/L) encompasses the level of concern for benthic invertebrates as it was

derived from an SSD that contained empirical data from the DIBP data set, read-across data from the DBP data set, as well as Web-ICE-derived predicted LC50s for several benthic species including worms (*Lumbriculus variegatus*), snails (*Physella gyrina*, *Lymnaea stagnalis*), and copepods (*Tigriopus japonicus*) (See Appendix B).

Chronic Aquatic Benthic Invertebrate Threshold

No chronic aquatic benthic invertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, analog data on chronic aquatic benthic invertebrate hazards from DBP exposure were used in a read-across to DIBP. The most sensitive hazard value resulted from a high-quality rated study on the midge (*Chironomus tentans*) (Call et al., 2001). In this study, a 10-day ChV for population loss of 1,143.3 mg DBP/kg dry sediment in medium-TOC sediments (4.80 percent) was identified. This study was conducted with low, medium, and high TOC sediments and toxicity was found to decrease with an increase in sediment TOC. This endpoint for deriving the COC using a medium-TOC was chosen because it is the closest to the assumed TOC level (4 percent) used in Point Source Calculator (EPA, 2019) to estimate DBP exposure in benthic organisms. At the LOEC identified in the study, 3,090 mg DBP/kg dry sediment, the midge population was reduced by 76.7 percent. Therefore, this endpoint was considered acceptable to derive a COC because of population-level relevance and a clear dose-response relationship. After dividing by an AF of 10, the chronic COC for benthic invertebrates is **114.3 mg/kg dry sediment.**

Aquatic Algae Threshold

No aquatic plant and algae studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, analog data on aquatic plant and algae hazards from DBP exposure were used in a read-across to DIBP. The most sensitive endpoint resulted from a medium-quality green algae (*Selenastrum capricornutum*) study (Adachi et al., 2006) with DBP concentrations ranging from 0.1 to 10 mg/L. In this study, algal population was found to be reduced at 1.0 mg/L. Thus, a 96-hour NOEC/LOEC of 0.1/1.0 mg/L, and a ChV of 0.316 mg/L was calculated. A clear dose-response relationship was observed and therefore this endpoint was considered acceptable to derive a COC. After dividing by an AF of 10, the COC for aquatic plants and algae is **31.6 µg/L**.

Table 6-1. Aquatic Environmental Hazard Threshold for DIBP

Receptor Group	Exposure Scenario	Phthalate	Hazard Threshold (COC)	Citation
Aquatic Vertebrates	Acute	DIBP and DBP	287 µg/L	SSD (See Section 3)
	Chronic	DBP	1.56 µg/L	(EAG Laboratories, 2018)
Aquatic Invertebrates	Acute	DIBP and DBP	287 µg/L	SSD (See Section 3)
	Chronic	DBP	12.23 µg/L	(Tagatz et al., 1983)
Benthic Invertebrates	Acute	DIBP and DBP	287 µg/L	SSD (See Section 3)
	Chronic	DBP	114.3 mg/kg dry sediment	(Call et al., 2001)
Aquatic Plants and Algae	NA	DBP	31.6 µg/L	(Adachi et al., 2006)

6.2 Terrestrial Species Hazard Values

Terrestrial mammal threshold

EPA reviewed two laboratory rodent studies as surrogates for hazards of DIBP to wild mammal populations (Saillenfait et al., 2008; Saillenfait et al., 2006). The most sensitive endpoint resulted from one study in which pregnant Sprague-Dawley rates were given DIBP at doses of 0 (olive oil), 250, 500, 750, and 1000 mg/kg/day via gavage. In both male and female fetuses, body weight was significantly lower (nine percent) at 500 mg/kg/day compared to controls, resulting in a gestational day 20 NOEC/LOEC of 250/500 mg/kg/day (Saillenfait et al., 2006). The ChV and thus the terrestrial mammal hazard threshold is **353 mg/kg/day.**

Terrestrial Invertebrate Threshold

No acceptable terrestrial invertebrate studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, analog data on terrestrial invertebrate hazards from DBP exposure were used in a read-across to DIBP. The most sensitive endpoint was found for the springtail (*Folsomia fimetaria*) with a 21-d EC10 of 14 mg DBP/kg dry soil for reduced reproduction (Jensen et al., 2001). This study was rated high quality. At the lowest concentration tested, 100 mg DBP/kg dry soil, reproduction was reduced by approximately 60% at the lowest concentration tested. This endpoint was considered acceptable to derive a hazard value because of population-level relevance and a clear dose-response relationship. Hazard values for soil invertebrates are calculated as the geometric mean of ChV, EC20, and EC10 values for apical endpoints such as mortality, reproduction, or growth.. Therefore, the hazard threshold for terrestrial invertebrates is **14 mg DBP/kg dry soil**.

Terrestrial Plant Threshold

No terrestrial plant studies were available for the quantitative assessment of potential hazards from DIBP exposure. Therefore, analog data on terrestrial plant hazards from DBP exposure were used in a read-across to DIBP. The hazard value used to derive a hazard threshold resulted from a high-quality rated study on bread wheat (*Triticum aestivum*) (Gao et al., 2019). In this study, a LOEL for reduction in leaf and root biomass in bread wheat seedlings at 10 mg/kg dry soil was observed. There was a clear dose-response observed, with biomass reduction increasing as the dose of DBP increased. At the highest dose (40 mg/kg), root and leaf biomass were reduced by 29.93 and 32.10 percent, respectively. Since the most sensitive endpoint in this study was an unbounded LOAEL, the actual threshold dose may have been lower than the lowest dose studied. However, no information was available in the study to adjust the value to account for this uncertainty. The hazard threshold for terrestrial plants for DBP derived from this study is 10 mg/kg dry soil.

Table 6-2. Terrestrial Environmental Hazard Threshold for DIBP

Receptor Group	Data Source	Hazard Threshold	Citation
Terrestrial Mammals	DIBP	353 mg/kg/day	(Saillenfait et al., 2006)
Terrestrial Invertebrates	DBP	14 mg DIBP/kg dry soil	(Tseng et al., 2013)
Terrestrial Plants	DBP	10 mg DBP/kg dry soil	(Gao et al., 2019)

7 ENVIRONMENTAL HAZARD ASSESSMENT CONCLUSIONS

EPA considered all reasonably available information identified through the systematic review process under TSCA to characterize environmental hazard endpoints for DIBP. The following bullets summarize the hazard values:

Aquatic species:

- DIBP had few reasonably available data to assess aquatic hazard.
- Analog data from DBP were used in a read-across to DIBP aquatic hazard.
- LC50 values from nine studies with exposures to DIBP and DBP in fish and aquatic invertebrates were used alongside Web-ICE hazard estimates to develop an SSD. The lower confidence interval of the HC05 was used as the COC and indicated that acute toxicity occurs at 287 µg/L for DIBP.
- The chronic aquatic vertebrate hazard threshold was derived from a read-across from DBP in which a three-generational reproductive study in Japanese medaka found significantly reduced body weight in F1 male fish after a 112-day exposure to DBP. The COC based on this study indicated that chronic toxicity in aquatic vertebrates occurs at 1.56 µg/L.
- The chronic aquatic invertebrate hazard threshold was derived from a read-across from DBP in which a 14-day exposure to DBP in the marine amphipod crustacean found a significant reduction in population abundance. The COC based on this study indicated that chronic toxicity in aquatic invertebrates occurs at 12.23 µg/L.
- The chronic aquatic benthic invertebrate hazard threshold was derived from a read-across from DBP in which a 10-day study on the midge identified a reduction in population at DBP concentrations in medium TOC. The COC based on this study indicated that chronic toxicity in chronic aquatic benthic invertebrates occurs at 114.3 mg/kg dry sediment.
- The aquatic plant and algae hazard threshold was derived from a read-across from DBP in which a 96-hour exposure to DBP in the green algae *Selenastrum capricornutum* found a significant reduction in population growth. The COC based on this study indicated that toxicity in aquatic plants and algae occurs at 31.6 µg/L.

Terrestrial Species:

- Terrestrial wildlife mammalian hazard data were not available for DIBP or the analog DBP, therefore studies in laboratory rats were used to derive hazard values. Empirical DIBP toxicity data for rats were used to estimate a hazard value for terrestrial mammals at 353 mg/kg-bw/day.
- The terrestrial invertebrate hazard threshold was derived from a read-across from DBP in which 21-day study in the springtail (*Folsomia fimetaria*) exposed to DBP via soil identified significant effects on reproduction. The hazard threshold based on this study indicated that toxicity in terrestrial invertebrates occurs at 14 mg DBP/kg dry soil.
- The terrestrial plant hazard threshold was derived from a read-across from DBP in which a reduction in leaf and root biomass in bread wheat seedlings exposed to DBP via soil was observed. The hazard threshold based on this study indicated that toxicity in terrestrial plant occurs at 10 mg DBP/kg dry soil.

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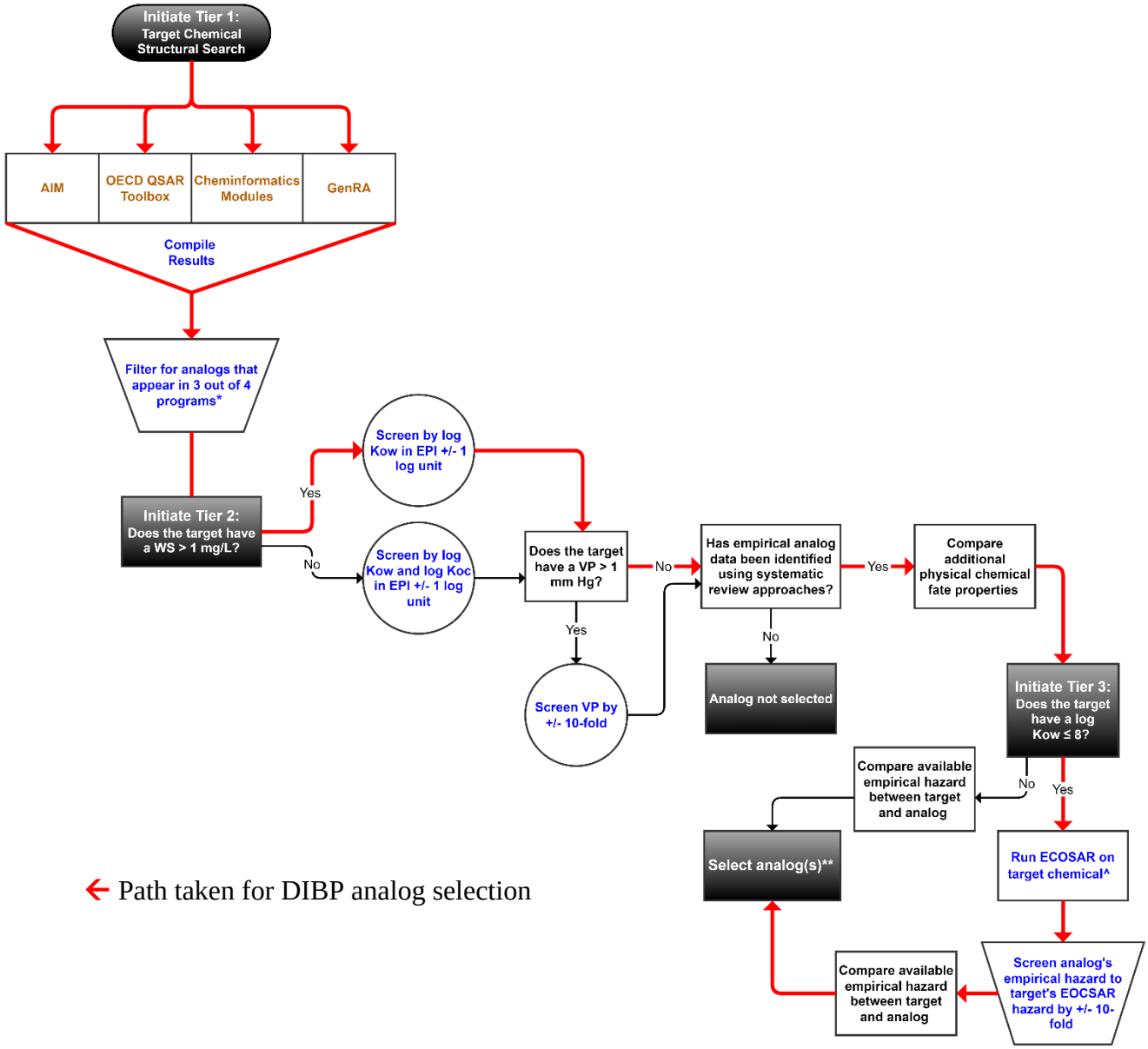
Appendix A Analog Selection for Environmental Hazard

DIBP environmental hazard data were only reasonably available for aquatic and benthic species exposed under acute durations with use of laboratory mammalian hazard data as surrogate for terrestrial mammalian wildlife hazard from DIBP exposure. No algal, chronic aquatic, chronic benthic, terrestrial plant, soil invertebrate, or avian hazard data were identified for DIBP. Additionally, the acute aquatic and acute benthic hazard data set for DIBP were limited to a single 24-hour water exposure in fathead minnows and a 96-hour water exposure in copepod *Nitocra spinipes*. Therefore, analog selection was performed to identify an appropriate analog to read across to DIBP to supplement the aquatic, benthic, terrestrial plant, soil invertebrate, and avian hazard data. Dibutyl phthalate (DBP) was selected as an analog for read-across of aquatic, benthic, and soil invertebrate hazard data based on excellent structural similarity, similar physical, chemical, environmental fate and transport behavior in water and sediment, and similar ecotoxicological behavior in aquatic taxa, including mechanistic hazard comparisons in the form of transcriptomic and metabolomic points of departure (Figure_Apx A-1). DBP was also selected for read-across of terrestrial plant and avian hazard, however, confidence in DBP as an analog for DIBP was decreased for read-across to these two taxa. This is because terrestrial plant and avian ecotoxicological similarity between DBP and DIBP could not be determined using the same means as in the aquatic, benthic, and soil invertebrate hazard analog selection, therefore the terrestrial plant and avian hazard read-across from DBP to DIBP was reliant upon similarity in structure as well as physical, chemical, environmental fate and transport. The DBP environmental hazard data to be used as analog data for DIBP received overall quality determinations of high or medium and are described in Section 3. The similarities between DIBP and analog DBP are described in detail below.



Figure_Apx A-1. Framework for DIBP Environmental Hazard Analog Selection.
*Criterion may be relaxed to results in fewer programs if no analogs are generated by one or more programs. ^ECOSAR acute and chronic toxicity predictions for vertebrates and invertebrates generated for chemicals with log $K_{OW} \leq 5$ and chronic toxicity predictions generated if log $K_{OW} \leq 8$, and algal toxicity predictions generated if log $K_{OW} \leq 6.4$ should the chemical meet the definition of an ECOSAR class. **Weight of scientific evidence and professional judgement involved in finalizing selection.

A.1 Structural Similarity

Structural similarity between DIBP and candidate analogs was assessed using two TSCA New Approach Methodologies (NAMs) (the Analog Identification Methodology (AIM) program and the Organisation of Economic Cooperative Development Quantitative Structure Activity Relationship [OECD QSAR] Toolbox) as well as two EPA Office of Research and Development tools (Generalized Read-Across [GenRA]) and the Search Module within the Cheminformatics Modules). These four programs provide

complementary methods of assessing structural similarity. There are several different methods for determining structural similarity. A fragment-based approach (*e.g.*, as implemented by AIM) searches for compounds with similar structural moieties or functional groups. EPA's TSCA New Chemicals Program utilizes CBI-AIM to identify analogs with data (including analogs with CBI). CBI information is not found in the public-facing version of AIM in order to protect business confidentiality, and CBI-AIM has undergone updates not found in the public-facing version of AIM. A structural identifier approach (*e.g.*, the Tanimoto coefficient) calculates a similarity coefficient based on molecular fingerprinting (Belford, 2023). Molecular fingerprinting approaches look at similarity in atomic pathway radius between the analog and target chemical substance (*e.g.*, Morgan fingerprint in GenRA which calculates a Jaccard similarity index). Some fingerprints may be better suited for certain characteristics and chemical classes. For example, substructure fingerprints like PubChem fingerprints perform best for small molecules such as drugs, while atom-pair fingerprints, which assigns values for each atom within a molecule and thus computes atom pairs based on these values, are preferable for large molecules. Some tools implement multiple methods for determining similarity. Regarding programs which generate indices, it has been noted that because the similarity value is dependent on the method applied, that these values should form a line of evidence rather than be utilized definitively (Pestana et al., 2021; Mellor et al., 2019).

AIM analogs were obtained using the Confidential Business Information (CBI) version of AIM and described as 1st or 2nd pass (only analogs not considered CBI are included in Table_Apx A-2). Tanimoto-based PubChem fingerprints were obtained in the OECD QSAR Toolbox (v4.4.1, 2020) using the Structure Similarity option and are presented as a range. Chemical Morgan Fingerprint scores were obtained in GenRA (v3.1) (limit of 100 analogs, no ToxRef filter). Tanimoto scores were obtained in the Cheminformatics Search Module using Similar analysis. AIM 1st and 2nd pass analogs were compiled with the top 100 analogs with indices greater than 0.5 generated from the OECD QSAR Toolbox and the Cheminformatics Search Module and indices greater than 0.1 generated from GenRA. These filtering criteria are displayed in Table_Apx A-1. Analogs that appeared in three out of four programs were identified as potential analog candidates (Figure_Apx A-1). Using these parameters, 25 analogs were identified as potentially suitable analog candidates for DIBP based on structural similarity (Table_Apx A-2). The results for structural comparison of DIBP to DBP (CASRN 84-74-2), diethylhexyl phthalate (DEHP, CASRN 117-81-7), diisodecyl phthalate (DIDP, CASRN 26761-40-0), and diisononyl phthalate (DINP, CASRN 28553-12-0) are further described below due to those analog candidates having completed data evaluation and extraction.

Table_Apx A-1. Structure Program Filtering Criteria

Program	Index	Filtering parameters
Analog Identification Methodology (AIM)	Fragment-based	1st or 2nd pass
OECD QSAR Toolbox	Tanimoto-based PubChem fingerprints	Top 100 analogs ≥ 0.5
Cheminformatics Search Module	Similarity-type: Tanimoto	Top 100 analogs with index ≥ 0.5
GenRA	Morgan Fingerprints	Top 100 analogs with index ≥ 0.1 (ToxRef data filter off)

DBP, DEHP, DINP, and DIDP were indicated as structurally similar to DIBP in AIM (analogs were 1st or 2nd pass), OECD QSAR Toolbox (PubChem features = 0.9-1), and the Cheminformatics Search Module (Tanimoto coefficient = 0.84-0.90) (Table_Apx A-2). Additionally, DBP and DEHP were indicated as structurally similar to DIBP in GenRA (Morgan Fingerprint = 0.48 and 0.51, respectively) (Table_Apx A-2). DBP was ultimately selected for read-across of aquatic, benthic, and terrestrial hazard to DIBP based on the additional lines of evidence (physical, chemical, and environmental fate and transport similarity and ecotoxicological similarity).

Table_Apx A-2. Structural Similarity between DIBP and Analog Candidates which met Filtering Criteria in at least 3 out of 4 Structure Programs

Chemical	CASRN	AIM	OECD QSAR Toolbox	Cheminformatics	GenRA	Count
DIBP (target)	84-69-5	Exact Match	1.00	1.00	1.00	4
Di(2-ethylhexyl) phthalate (DEHP)[a]	117-81-7	1st pass	0.90-1.00	0.90	0.51	4
Bis(2-propylheptyl) phthalate	53306-54-0	1st pass	0.90-1.00	0.90	0.48	4
Butyl 2-ethylhexyl phthalate	85-69-8	1st pass	0.90-1.00	0.90	–	3
Isoamyl phthalate	605-50-5	1st pass	0.90-1.00	0.90	0.58	4
Diisodecyl phthalate (DIDP)[a]	26761-40-0	1st pass	0.90-1.00	0.84	–	3
Diisooctyl phthalate	27554-26-3	1st pass	0.90-1.00	0.84	–	3
Diisononyl phthalate (DINP)[a]	28553-12-0	1st pass	0.90-1.00	0.84	–	3
Di(2-ethyl-4-methylpentyl) phthalate	2229-55-2	1st pass	–	0.90	0.60	3
Di-n-propylphthalate	131-16-8	2nd pass	0.90-1.00	0.95	0.51	4
Dibutyl 1,2-benzenedicarboxylate (DBP)[a]	84-74-2	2nd pass	0.90-1.00	0.90	0.48	4
Diethyl phthalate	84-66-2	2nd pass	0.90-1.00	0.89	0.56	4

Chemical	CASRN	AIM	OECD QSAR Toolbox	Cheminformatics	GenRA	Count
Dipentyl phthalate	131-18-0	2nd pass	0.90-1.00	0.88		3
Dihexyl phthalate	84-75-3	2nd pass	0.90-1.00	0.88		3
Di-n-octyl phthalate	117-84-0	2nd pass	0.90-1.00	0.88		3
Ditridecyl phthalate	119-06-2	2nd pass	0.90-1.00	0.88	–	3
Didodecyl phthalate	2432-90-8	2nd pass	0.90-1.00	0.88	–	3
Diundecyl phthalate	3648-20-2	2nd pass	0.90-1.00	0.88	–	3
Diheptyl phthalate	3648-21-3	2nd pass	0.90-1.00	0.88	–	3
Dinonyl phthalate	84-76-4	2nd pass	0.90-1.00	0.88	–	3
Didecyl phthalate	84-77-5	2nd pass	0.90-1.00	0.88	–	3
Dimethyl phthalate	131-11-3	2nd pass	0.90-1.00	0.84	–	3
Isobutyl benzoate	120-50-3	2nd pass	–	0.92	0.51	3
Terephthalic acid, diisobutyl ester	18699-48-4	2nd pass	–	0.92	0.50	3
Di(2-methoxyethyl) phthalate	117-82-8	–	0.90-1.00	0.87	0.49	3
Cyclohexyl 2-isobutyl phthalate	5334-09-8	–	0.90-1.00	0.84	0.61	3

[a] Analogs which have completed data evaluation and extraction are bolded.

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

DIBP analog candidates from the structural similarity analysis were preliminarily screened based on similarity in log octanol-water partition coefficient (log K_{OW}) obtained using EPI Suite™ (Figure_Apx

A-1). For this screening step, DIBP, DBP, DEHP, DIDP, and DINP values were obtained from their respective final scope documents (Abt Associates, 2021; U.S. EPA, 2021b, 2020a, b, c). Analog candidates with log K_{OW} values within one log unit relative to DIBP were considered potentially suitable analog candidates for DIBP. This preliminary screening analysis narrowed the analog candidate list from 25 candidate analogs to 3 candidate analogs (Table_Apx A-3). One of the three candidate analogs was DBP. A more expansive analysis of physical, chemical, environmental fate and transport similarities between DIBP and DBP was conducted because DBP's hazard data had completed data evaluation and extraction (Figure_Apx A-1).

Table_Apx A-3. Analog Candidates with Similar log K_{OW} values to that of DIBP

Chemical	CASRN	Log K_{OW}
DIBP (target)	84-69-5	4.34
DBP	84-74-2	4.53
Diisobutyl terephthalate	18699-48-4	4.46[a]
Cyclohexyl 2-isobutyl phthalate	5334-09-8	5.33[a]

[a] Values predicted using EPI Suite™
[b] Analogs which have completed data evaluation and extraction are bolded.

Physical, chemical, and environmental fate and transport similarities between DIBP and DBP were assessed based on properties relevant to the to the aquatic, benthic, and soil compartments and are shown in Table_Apx A-4. Physical, chemical, and environmental fate and transport values for DIBP and DBP are specified in the *Draft Fate & Physical Chemistry Assessment for Diisobutyl Phthalate (DIBP)* (U.S. EPA, 2024e), *Draft Fate Assessment for Diisobutyl Phthlate (DIBP)* (U.S. EPA, 2024d), *Draft Fate & Physical Chemistry Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2024c). DIBP and DBP water solubilities are similar in value (6.2 mg/L and 11.2 mg/L, respectively) indicating both target and analog are fairly insoluble in water. The selected octanol-water partition coefficients (log K_{OW}) are very similar in value (4.34 and 4.5 for DIBP and DBP, respectively), indicating relatively low affinity for water and higher sorption potential to soils and sediments for target and analog. Degradation of DIBP and DBP in both water and sediment is also similar, with almost complete aerobic biodegradation in water within 4 weeks and slower anaerobic degradation in sediment (Table_Apx A-4). Both DIBP and DBP would biodegrade in water before hydrolyzing. Similar biodegradation rates between target and analog can increase confidence when considering read across of chronic hazard. The values for DIBP's and DBP's log organic carbon-water partition coefficients indicate both target and analog will be preferentially bound to sediment or soil than exist in the water. Bioaccumulation potential of DBP in aquatic organisms is slightly higher than for DIBP by one to two orders of magnitude (Table_Apx A-4), however both phthalates have fairly low bioconcentration and bioaccumulation potential. An almost identical freshwater magnification factor of less than 1 was derived across 18 species for DIBP and DBP indicating that both phthalates do not biomagnify up the trophic levels (Table_Apx A-4). Regarding fate in terrestrial species, bioconcentration of DIBP and DBP in various terrestrial plants is low (0.13-2.23 and 0.02-9.32, respectively, Table_Apx A-4). Almost identical uptake behavior was noted in ants covered with either 2,000 ng DIBP or DBP (Lenoir et al., 2014). DIBP's and DBP's vapor pressures are very low (4.76×10^{-5} mmHg and 2.01×10^{-5} mmHg, respectively) as are their Henry's law constants

(1.83×10^{-7} atm-m^3/mol and 1.81×10^{-6} atm-m^3/mol, respectively), indicating both chemicals are not readily volatile. Both phthalates exist as a liquid at room temperature and have similar molecular weights. The similarity in the properties described in Table_Apx A-4 support the ability to read across DBP aquatic and benthic hazard as well as terrestrial (plant, soil invertebrate, and avian) hazard to supplement the DIBP environmental hazard data set.

Table_Apx A-4. Comparison of DIBP and DBP for Several Physical and Chemical and Environmental Fate Properties Relevant to Water, Sediment, and Soil

Property	DIBP (target)	DBP
Water Solubility	6.2 mg/L	11.2 mg/L
Log K_{OW}	4.34	4.5
Log K_{OC}	2.67 (2.50 – 2.86)	3.69 (3.14 – 3.94)
Hydrolysis ($t_{1/2}$)	5.3 yr (pH 7); 195 days (pH 8)	3.43 yr (pH 7); 125 days (pH 8)
Aerobic biodegradation in water	42 to 98% in 28 days	68.3 to >99% after 28 days
Anaerobic biodegradation in sediment	0 – 30% after 56 days	$t_{1/2}$ = 14.4 days
BCF	30.2 L/kg wet weight (estimated)	2.9 – 176 (experimental, various aquatic species)
BAF (aquatic)	30.2 L/kg wet weight (estimated)	100 – 1,259 (experimental, various fish species), 159 (estimated)
FWMF (aquatic)	0.81 (18 marine species)	0.70 (18 marine species)
BCF (plants)	0.13-2.23 (onion, celery, pepper, tomato, bitter gourd, eggplant, and long podded cowpea)	0.02-9.32 (rice, radish, wheat, maize, strawberry, carrot, lettuce, wetland grasses)
Henry's Law Constant (atm-m^3/mol)	1.83×10^{-7}	1.81×10^{-6}
Vapor Pressure (mmHg)	4.76×10^{-5}	2.01×10^{-5}
Molecular Weight	278.35 g/mol	278.35 g/mol
Physical state of the chemical	Clear Viscous Liquid	Clear Oily Liquid

A.3 Ecotoxicological Similarity

Ecotoxicological similarity between DIBP and DBP was assessed based on two lines of evidence: the first line of evidence was a comparison of the analog's empirical hazard data to corresponding toxicity predictions of the target and the second line of evidence was a comparison of several points of departure derived for DIBP and DBP following acute exposures to fathead minnow and copepod *Nitocra spinipes* (Bencic et al., 2024; Linden et al., 1979). Although less relevant than hazard obtained from sediment exposures, toxicological similarity in empirical hazard evidence for aquatic invertebrates exposed to

DIBP and DBP in water was also assessed to determine suitability of DBP for read-across of soil invertebrate hazard data to DIBP. Ecotoxicological comparisons made for algae helped support the read-across for terrestrial plant hazard, while acknowledging the differences between nonvascular aquatic biota and vascular terrestrial plants. DIBP toxicity predictions for acute and chronic exposure to fish, aquatic invertebrates, and green algae were generated using ECOSAR v2.2. Empirical hazard data used in the following comparisons were from studies with overall quality determinations of high and medium. The ecotoxicological similarity line of evidence had uncertainty in supporting the avian hazard read-across from DBP to DIBP due to a lack of predictive tools for assessing this hazard, therefore less confidence was had in the avian read-across.

Comparison of the analog empirical hazard data to corresponding ECOSAR toxicity predictions for DIBP shows agreement of hazard values well within 10-fold (Figure_Apx A-1, Table_Apx A-5). Average ratio of empirical DBP aquatic hazard data to predicted DIBP hazard values is 1.3 ± 0.20 (standard error) (Table_Apx A-5) which indicates very similar ecotoxicological behavior between DBP and DIBP when aquatic vertebrates, aquatic invertebrates, and algae are exposed under acute and chronic conditions and that DBP is an appropriate analog for DIBP. An additional comparison based on DIBP and DBP empirical hazard from the same studies also indicate ecotoxicological similarity between DIBP and DBP. Transcriptomic, metabolomic, and swimming behavior points of departure as well as LC50 values were derived for DIBP and DBP following a 24-hour exposure to fathead minnow (Bencic et al., 2024). In a second study, 96-hour LC50 values were derived for benthic invertebrate *N. spinipes* exposed to DIBP and DBP (Linden et al., 1979). Although the DIBP and DBP hazard values are within 10-fold of each other and suggest general agreement, the lower hazard values for DBP when compared to DIBP indicate the analog data is protective of the target when both phthalates are tested in the same study across two aquatic taxa, with average ratio of DBP hazard to DIBP hazard 0.38 ± 0.11 (standard error) (Table_Apx A-6). These comparisons support the appropriateness to read-across DBP aquatic and benthic hazard data to DIBP. Ecotoxicological similarity for a soil invertebrate hazard read-across is inferred by the aquatic and benthic invertebrate toxicity comparisons made between DIBP and DBP, similar to the read-across approach used for other phthalates (U.S. EPA, 2024g).

Table_Apx A-5. Ecotoxicological similarity in aquatic taxa exposed to DIBP (predicted hazard) and DBP (empirical hazard)

Taxa	Duration	Endpoint	DIBP	DBP	Ratio of DBP toxicity to DIBP toxicity
			Predicted hazard (mg/L)[a]	Empirical hazard (mg/L)[a]	
Fish	96-h	LC50	1.30	1.08[b]	0.8
Daphnid	48-h	LC50	2.57	3.44[c]	1.3
Mysid	96-h	LC50	0.98	0.61[d]	0.6
Green Algae	96-h	EC50	0.82	1.12[e]	1.4
Fish		ChV	0.11	0.10[f]	0.9
Daphnid		ChV	0.54	1.14[g]	2.1
Green Algae		ChV	0.31	0.56[h]	1.8

Taxa	Duration	Endpoint	DIBP	DBP	Ratio of DBP toxicity to DIBP toxicity
			Predicted hazard (mg/L)[a]	Empirical hazard (mg/L)[a]	
Average fold-hazard DBP:DIBP					**1.3 ± 0.20**

[a] Hazard values, including empirical hazard values used to calculate a geometric mean, were limited to those at or below the phthlate-specific water solubility limit.
[b] Value for DBP represents a geometric mean of 96-hour fish (*Lepomis macrochirus*, *Pimephales promelas*, *Oncorhynchus mykiss*) LC50 data from (Smithers Viscient, 2018; Adams et al., 1995; DeFoe et al., 1990; McCarthy and Whitmore, 1985; EG&G Bionomics, 1983b; Buccafusco et al., 1981).
[c] Value for DBP represents a geometric mean of 48-hour *Daphnia magna* LC50 and 48-hour *Daphnia magna* immobilization EC50 data from (Shen et al., 2019; Wei et al., 2018; Adams et al., 1995; McCarthy and Whitmore, 1985).
[d] Value for DBP represents a geometric mean of 96-hour *Americamysis bahia* LC50 data from (Smithers Viscient, 2018; Adams et al., 1995; DeFoe et al., 1990; McCarthy and Whitmore, 1985; EG&G Bionomics, 1983b; Buccafusco et al., 1981).
[e] Value for DBP represents a geometric mean of 96-hour green algae (*Selenastrum capricornutum* and *Chlorella pyrenoidosa*) EC50 data from (Gu et al., 2017; Adams et al., 1995).
[f] Value for DBP represents a geometric mean of fish (*Lepomis macrochirus*, *Pimephales promelas*, *Oncorhynchus mykiss*, *Oryzias latipes*) NOEC/LOEC pairs for Mortality, Reproduction, and Development/Growth endpoints from (Smithers Viscient, 2018; Adams et al., 1995; DeFoe et al., 1990; McCarthy and Whitmore, 1985; EG&G Bionomics, 1983b; Buccafusco et al., 1981). Exposures and study durations were a minimum of 13 days.
[g] Value for DBP represents a geometric mean of *Daphnia magna* NOEC/LOEC pairs for Mortality, Reproduction, and Development/Growth endpoints from (Seyoum and Pradhan, 2019; Wei et al., 2018; Rhodes et al., 1995; DeFoe et al., 1990; Springborn Bionomics, 1984). Exposures and study durations were a minimum of 1 week and 2 weeks, respectively.
[h] Value for DBP represents a geometric mean of green algae (*Selenastrum capricornutum*) NOEC/LOEC pairs for Development/Growth endpoints from (Adachi et al., 2006).

Table_Apx A-6. Comparison of DIBP and DBP Points of Departure and LC50 Values in Fathead Minnow Exposed for 24-hours, and LC50 Values in *Nitocra spinipes* Exposed for 96-hours

Species	Outcome	Endpoint	DIBP Hazard (mg/L)	DBP Hazard (mg/L)	Ratio of DBP toxicity to DIBP toxicity
Fathead minnow [a]	Transcriptomics	POD	0.87	0.12	0.14
Fathead minnow [a]	Metabolomics	POD	0.15	0.11	0.73
Fathead minnow [a]	Swimming behavior	POD	0.90	0.24	0.27
Fathead minnow [a]	Mortality	LC50	5.30	1.02	0.19
Nitocra spinipes [b]	Mortality	LC50	3.0	1.7	0.57

Species	Outcome	Endpoint	DIBP Hazard (mg/L)	DBP Hazard (mg/L)	Ratio of DBP toxicity to DIBP toxicity
Average fold-hazard DBP:DIBP					**0.38 ± 0.11**

[a] Data are based on measured concentrations from (Bencic et al., 2024).
[b] Data are based on measured concentrations from (Linden et al., 1979).

A.4 Read-Across Weight of the Scientific Evidence and Conclusions

DIBP presented with minimal acute aquatic and benthic hazard data, no chronic aquatic or chronic benthic hazard data, no algal hazard data, and no terrestrial plant, soil invertebrate, or avian hazard data. Analog selection was carried out to address these data gaps. Several phthalates of interest (DBP, DEHP, DIDP, and DINP) were indicated as structurally similar to DIBP. A screening by $\log K_{OW}$ values and further comparison of additional physical, chemical, and environmental fate and transport properties indicated that DBP, which is data-rich for aquatic and benthic hazard, was very similar to DIBP. A comparison of available DBP empirical hazard data to corresponding DIBP toxicity predictions for aquatic taxa showed high concordance between analog and target hazard. A second toxicity comparison was made in fathead minnow and copepod *N. spinipes* exposed to either DBP or DIBP for 24 hours (fathead minnow) or 96 hours (*N. spinipes*); DBP points of departure and LC50 in fathead minnow were within 10-fold of and protective of DIBP points of departure and LC50. This was also the case for comparison of the DIBP and DBP LC50 values in *N. spinipes*. Ecotoxicological similarity for a soil invertebrate hazard read-across is inferred by the aquatic and benthic invertebrate toxicity comparisons made between DIBP and DBP, although this inference has slightly greater uncertainty than when it was made in a previous read-across (U.S. EPA, 2024g). The greater uncertainty is due to a lack to DIBP sediment exposure data with which to compare to DBP sediment exposure data as a more relevant ecotoxicological comparison for a soil invertebrate hazard read-across. Because of a lack of predictive tools to assess ecotoxicological similarity in terrestrial plants and birds, the read-across for these two taxa was based largely on the physical, chemical, environmental fate and transport agreement between DIBP and DBP as well as their close structural similarity. Bioconcentration in terrestrial plants was very similar between DIBP and DBP which increased confidence that both phthalates would behave similarly in terrestrial plants. Ecotoxicological similarity in algae also helped support the read-across of DBP terrestrial plant hazard data to DIBP. Uncertainty in establishing ecotoxicological similarity for these two taxa decreased confidence in the read-across from DBP to DIBP for terrestrial plant and avian hazard, whereas the aquatic hazard read-across had high confidence, followed by moderate confidence in the benthic and soil invertebrate hazard read-across from DBP to DIBP. Looking across the multiple lines of evidence (structural, physical/chemical, ecotoxicological), DBP is an appropriate analog with high and medium quality aquatic, benthic, and terrestrial hazard data to be used in a read-across to DIBP.

Appendix B Species Sensitivity Distribution for Acute Aquatic Hazard

The SSD Toolbox (v1.1) is a resource created by EPA's Office of Research and Development (ORD) that can fit SSDs to environmental hazard data (Etterson, 2020). It runs on Matlab 2018b (9.5) for Windows 64 bit. For this DIBP risk evaluation, EPA created one SSD with the SSD Toolbox 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 that measured mortality for aquatic vertebrates and invertebrates.

Table_Apx B-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 72 additional species were added (Appendix B). Hazard predictions were limited to at or below the limit of water solubility for DIBP (6.2 mg/L). With this data set, the SSD Toolbox was used to apply a variety of algorithms to fit and visualize SSDs with different distributions.

The Web-ICE application was developed by EPA and collaborators to provide interspecies extrapolation models for acute toxicity (Raimondo, 2010). These models estimate the acute toxicity (LC50/LD50) of a chemical to a species, genus, or family with no test data (the predicted taxon) from the known toxicity of the chemical to a species with test data (the commonly tested surrogate species). Web-ICE models are log-linear least square regressions of the relationship between surrogate and predicted taxon based on a database of acute toxicity values. The model returns median effect or lethal water concentrations for aquatic species (EC50/LC50). Separate acute toxicity databases are maintained for aquatic animals (vertebrates and invertebrates), aquatic plants (algae), and terrestrial wildlife (birds and mammals), with 1,440 models for aquatic taxa and 852 models for wildlife taxa in Web-ICE version 3.3 (Willming et al., 2016). Open-ended toxicity values (*i.e.*, >100 mg/kg or <100 mg/kg) and duplicate records among multiple sources are not included in any of the databases. The aquatic animal database within Web-ICE is composed of 48- or 96-hour EC50/LC50 values based on death or immobility. This database is described in detail in the Aquatic Database Documentation found on the Download Model Data page of Web-ICE and describes the data sources, normalization, and quality and standardization criteria (*e.g.*, data filters) for data used in the models. Data used in model development adhered to standard acute toxicity test condition requirements of the ASTM International (ASTM, 2014) and the U.S. EPA Office of Chemical Safety and Pollution Prevention (*e.g.* (U.S. EPA, 2016a)).

EPA used empirical DIBP data for the harpacticoid copepod and the fathead minnow and DBP data for bluegill, opossum shrimp, rainbow trout, zebrafish, the midge (*Paratanytarsus parthenogeneticus* and *Chironomus plumosus*), and the water flea as surrogate species to predict LC50 toxicity values using the Web-ICE application (U.S. EPA, 2024h). The Web-ICE model estimated toxicity values for 72 species. For model validation, the model results are then screened by the following quality standards to ensure confidence in the model predictions. If a predicted species did not meet all the quality criteria below, the species was eliminated from the data set (Willming et al., 2016).

- High R^2 (≥0.6)
  - The proportion of the data variance that is explained by the model. The closer the R^2 value is to one, the more robust the model is in describing the relationship between the predicted and surrogate taxa.
- Low Mean Square Error (MSE; ≤0.95)

- An unbiased estimator of the variance of the regression line.
- High slope (≥0.6)
  - The regression coefficient represents the change in log10 value of the predicted taxon toxicity for every change in log10 value of the surrogate species toxicity.
- Narrow 95 percent confidence intervals
  - One order of magnitude between lower and upper limit

The toxicity data were then used to calculate the distribution of species sensitivity through the SSD toolbox (Etterson, 2020). The SSD Toolbox's output contained several methods for choosing an appropriate distribution and fitting method, including goodness-of-fit, standard error, and sample-size corrected Akaike Information Criterion (BIC_c, (Burnham and Anderson, 2002)). Most *P* values for goodness-of-fit were above 0.05, showing no evidence for lack of fit. The distribution and model with the lowest BIC_c value, and therefore the best fit for the data was the Metropolis Hastings: Logistic (Figure_Apx B-1)

Table_Apx B-1. Species Sensitivity Distribution (SSD) Model Input for Acute Exposure Toxicity in Aquatic Vertebrates and Invertebrates – Empirical Data

Genus	Species	Acute Toxicity Value LC_{50} (µg/L)	Reference
Americamysis	*bahia*	612	(EG&G Bionomics, 1984a)
Danio	*rerio*	630	(Chen et al., 2014)
Lepomis	*macrochirus*	788	(Adams et al., 1995; EG&G Bionomics, 1983b; Buccafusco et al., 1981)
Oncorynchus	*mykiss*	1497	(EnviroSystem, 1991; EG&G Bionomics, 1983a)
Nitocra	***spinipes***	**3000**	**(**Linden et al., 1979**)**
Daphnia	*magna*	3443	(Wei et al., 2018; McCarthy and Whitmore, 1985)
Chironomus	*plumosus*	4648	(Streufort, 1978)
Pimephales	***promelas***	**5300**	**(**Bencic et al., 2024**)**
Paratanytarsus	*parthenogeneticus*	5800	(EG&G Bionomics, 1984b)
Bolded value indicates DIBP empirical data. Unbolded value indicates DBP empirical data.			

Table_Apx B-2. Species Sensitivity Distribution (SSD) Model Input for Acute Exposure Toxicity

in Aquatic Vertebrates and Invertebrates – Web-ICE Data

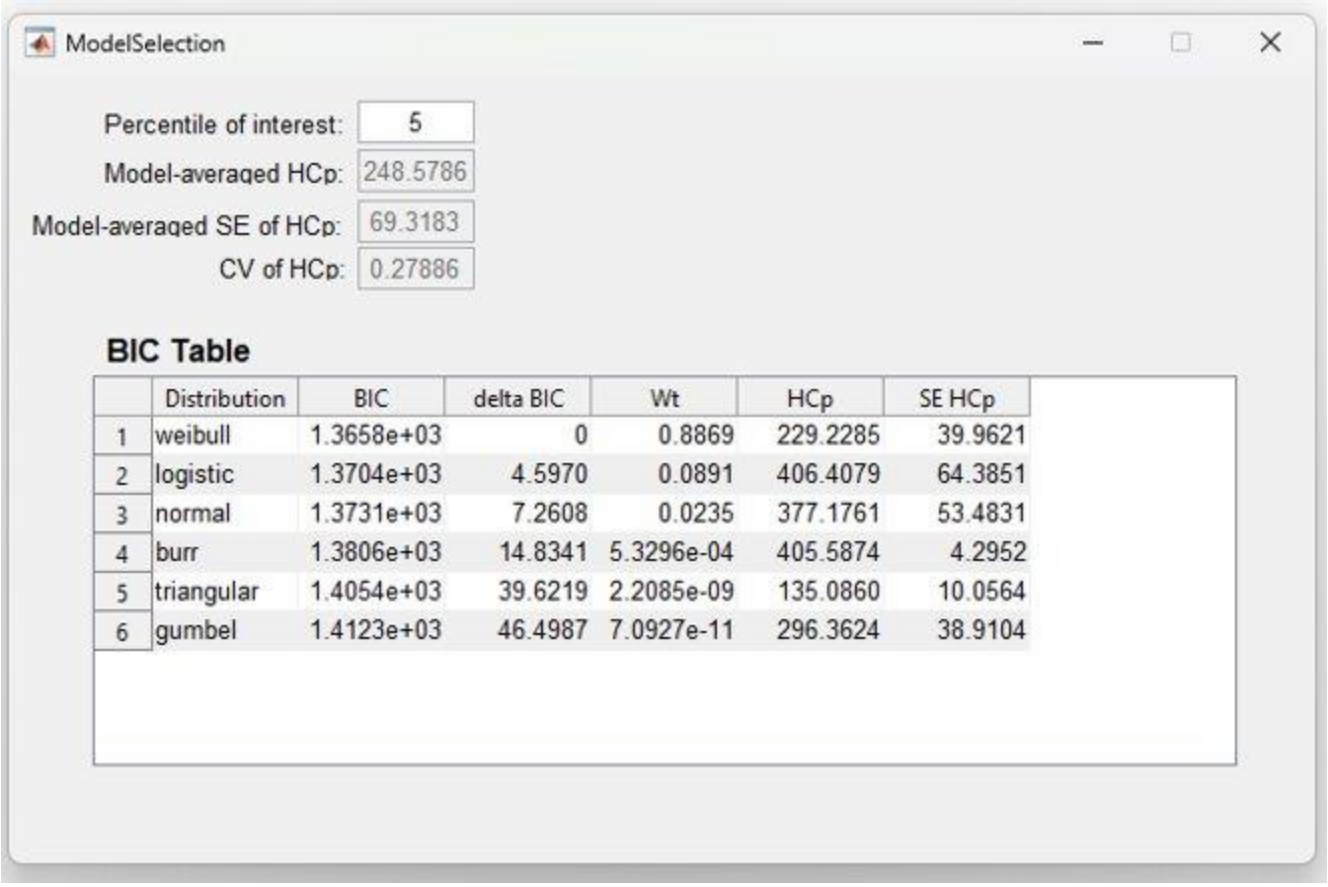
Genus	Species	Acute Toxicity Value LC_{50} (µg/L)
Gammarus	*pseudolimnaeus*	333
Gammarus	*pseudolimnaeus*	3051
Menidia	*peninsulae*	318
Catostomus	*commersonii*	537
Menidia	*menidia*	495
Caecidotea	*brevicauda*	611
Caecidotea	*brevicauda*	756
Perca	*flavescens*	520
Perca	*flavescens*	1413
Allorchestes	*compressa*	2026
Allorchestes	*compressa*	289
Allorchestes	*compressa*	2150
Jordanella	*floridae*	924
Sander	*vitreus*	480
Crassostrea	*virginica*	2036
Crassostrea	*virginica*	379
Crassostrea	*virginica*	243
Oncorhynchus	*kisutch*	1476
Oncorhynchus	*kisutch*	445
Oncorhynchus	*kisutch*	2125
Oncorhynchus	*clarkii*	1746
Oncorhynchus	*clarkii*	924
Oncorhynchus	*clarkii*	1480
Salvelinus	*namaycush*	813
Salvelinus	*namaycush*	637
Salvelinus	*namaycush*	1167

Genus	Species	Acute Toxicity Value LC_{50} (µg/L)
Salmo	*salar*	480
Salmo	*salar*	1394
Lumbriculus	*variegatus*	6099
Salvelinus	*fontinalis*	1321
Salvelinus	*fontinalis*	559
Salvelinus	*fontinalis*	1485
Oreochromis	*mossambicus*	3763
Oreochromis	*mossambicus*	1579
Oreochromis	*niloticus*	967
Micropterus	*salmoides*	766
Micropterus	*salmoides*	1089
Oncorhynchus	*tshawytscha*	1779
Simocephalus	*serrulatus*	1979
Amblema	*plicata*	846
Cyprinus	*carpio*	5624
Cyprinus	*carpio*	1260
Cyprinus	*carpio*	3020
Acipenser	*brevirostrum*	1297
Cyprinodon	*variegatus*	3672
Cyprinodon	*variegatus*	1224
Cyprinodon	*variegatus*	2602
Cyprinodon	*variegatus*	553
Xyrauchen	*texanus*	2437
Oncorhynchus	*gilae*	1365
Lasmigona	*subviridis*	1996
Salmo	*trutta*	350
Salmo	*trutta*	1553

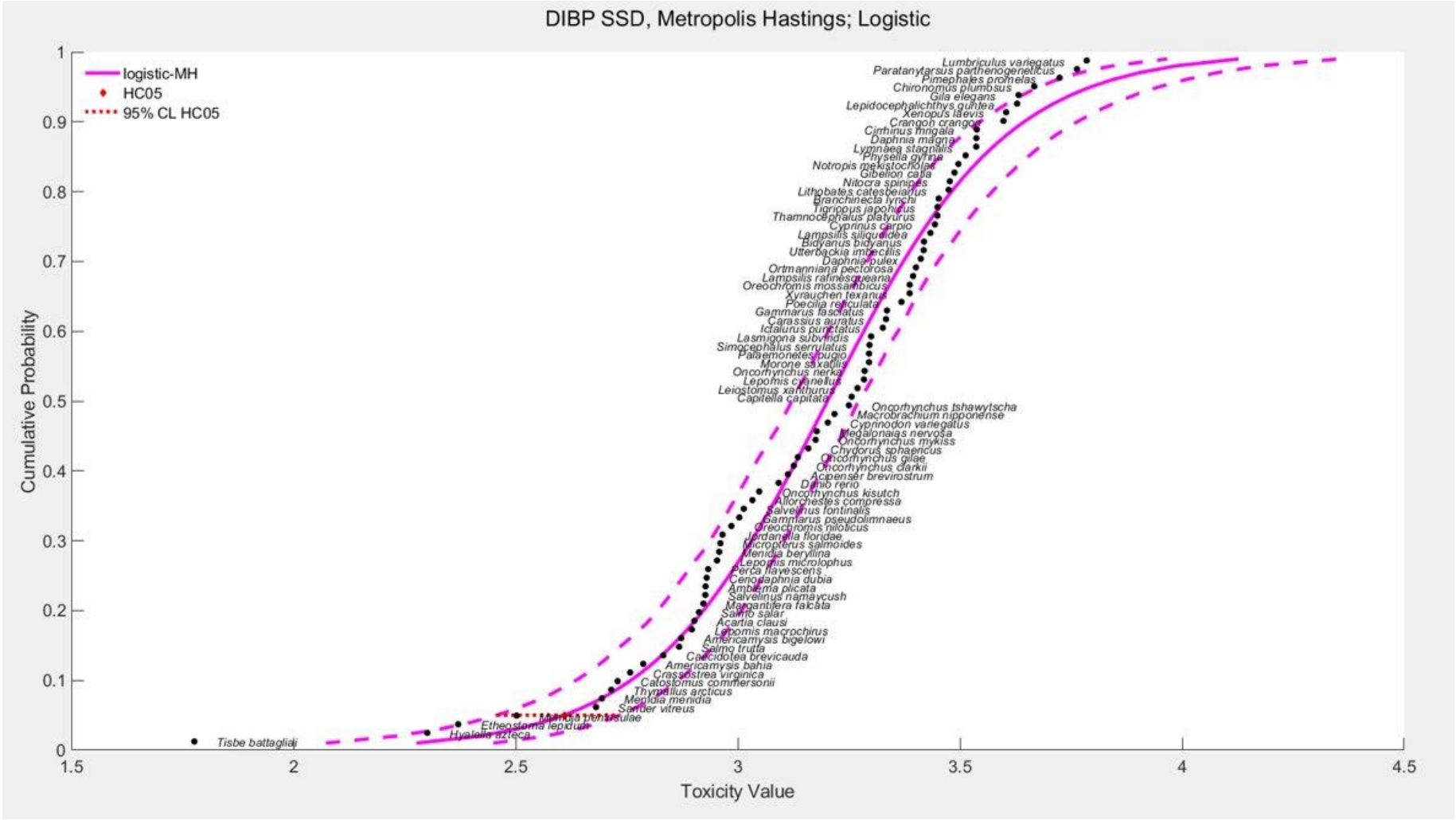
Genus	Species	Acute Toxicity Value LC_{50} (µg/L)
Poecilia	*reticulata*	3310
Poecilia	*reticulata*	1204
Poecilia	*reticulata*	3199
Menidia	*beryllina*	908
Ictalurus	*punctatus*	5022
Ictalurus	*punctatus*	1244
Ictalurus	*punctatus*	2585
Ictalurus	*punctatus*	1252
Megalonaias	*nervosa*	1505
Lepomis	*cyanellus*	1279
Lepomis	*cyanellus*	2890
Lepomis	*microlophus*	898
Lithobates	*catesbeianus*	5832
Lithobates	*catesbeianus*	1131
Lithobates	*catesbeianus*	4024
Oncorhynchus	*nerka*	1930
Utterbackia	*imbecillis*	2619
Carassius	*auratus*	5103
Carassius	*auratus*	5103
Carassius	*auratus*	1143
Ceriodaphnia	*dubia*	325
Ceriodaphnia	*dubia*	2227
Thamnocephalus	*platyurus*	2814
Margaritifera	*falcata*	1651
Margaritifera	*falcata*	289
Daphnia	*pulex*	2582
Branchinecta	*lynchi*	2834

Genus	Species	Acute Toxicity Value LC_{50} (µg/L)
Lampsilis	*siliquoidea*	2713
Lampsilis	*rafinesqueana*	2481
Notropis	*mekistocholas*	3137
Gammarus	*fasciatus*	2166
Tigriopus	*japonicus*	2816
Lymnaea	*stagnalis*	3440
Acartia	*clausi*	799
Americamysis	*bigelowi*	638
Americamysis	*bigelowi*	873
Bidyanus	*bidyanus*	2642
Capitella	*capitata*	1804
Chydorus	*sphaericus*	1441
Cirrhinus	*mrigala*	3450
Crangon	*crangon*	3961
Danio	*rerio*	5006
Danio	*rerio*	393
Danio	*rerio*	1881
Etheostoma	*lepidum*	235
Gibelion	*catla*	5167
Gibelion	*catla*	1829
Gila	*elegans*	4283
Hyalella	*azteca*	67
Hyalella	*azteca*	598
Leiostomus	*xanthurus*	1859
Lepidocephalichthys	*guntea*	4252
Macrobrachium	*nipponense*	1651
Morone	*saxatilis*	1974

Genus	Species	Acute Toxicity Value LC_{50} (µg/L)
Ortmanniana	*pectorosa*	2518
Palaemonetes	*pugio*	1975
Physella	*gyrina*	3256
Thymallus	*arcticus*	519
Tisbe	*battagliai*	60
Xenopus	*laevis*	4017



Figure_Apx B-1. SSD Toolbox Model Fit Parameters



Figure_Apx B-2. Species Sensitivity Distribution (SSD) for Acute DIBP Toxicity to Aquatic Vertebrates and Invertebrates

Appendix C Environmental Hazard Details

C.1 Evidence Integration

Data integration includes analysis, synthesis, and integration of information for the draft risk evaluation. During data integration, EPA considers quality, consistency, relevancy, coherence, and biological plausibility to make final conclusions regarding the weight of the scientific evidence. As stated in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021a), data integration involves transparently discussing the significant issues, strengths, and limitations as well as the uncertainties of the reasonably available information and the major points of interpretation.

The general analytical approaches for integrating evidence for environmental hazard is discussed in Section 7.4 of the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021a).

The organization and approach to integrating hazard evidence is determined by the reasonably available evidence regarding routes of exposure, exposure media, duration of exposure, taxa, metabolism and distribution, effects evaluated, the number of studies pertaining to each effect, as well as the results of the data quality evaluation.

The environmental hazard integration is organized around effects to aquatic and terrestrial organisms as well as the respective environmental compartments (*e.g.*, pelagic, benthic, soil). Environmental hazard assessment may be complex based on the considerations of the quantity, relevance, and quality of the available evidence.

For DIBP, environmental hazard data from toxicology studies identified during systematic review have used evidence that characterizes apical endpoints; that is, endpoints that could have population-level effects such as reproduction, growth, and/or mortality. Additionally, mechanistic data that can be linked to apical endpoints will add to the weight of the scientific evidence supporting hazard thresholds.

C.1.1 Weight of the Scientific Evidence

After calculating the hazard thresholds that were carried forward to characterize risk, a narrative describing the weight of the scientific evidence and uncertainties was completed to support EPA's decisions. The weight of the 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 the 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 (U.S. EPA, 2021a) guides the application of strength-of-evidence judgments for environmental hazard effect within a given evidence stream and were adapted from Table 7-10 of the *2021 Draft Systematic Review Protocol* (U.S. EPA, 2021a)

EPA used the strength-of-evidence and uncertainties from (U.S. EPA, 2021a) for the hazard assessment to qualitatively rank the overall confidence using evidence (Table_Apx C-1) for environmental hazard. Confidence levels of robust (+ + +), moderate (+ +), slight (+), or indeterminant are assigned for each evidence property that corresponds to the evidence considerations (U.S. EPA, 2021a). 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 determinations 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 the 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.

Confidence Levels

- Robust (+++) confidence suggests thorough understanding of the scientific evidence and uncertainties. The supporting weight of the 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 the 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.

C.1.2 Data Integration Considerations Applied to Aquatic and Terrestrial Hazard Representing the DIBP Environmental Hazard Database

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 on the basis of causal inferences.
    - Modeling assumptions may be simplified representations of reality.

Table_Apx C-1 summarizes the weight of the 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 C-1. 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; see U.S. EPA (2005b) decreases strength.) • 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.