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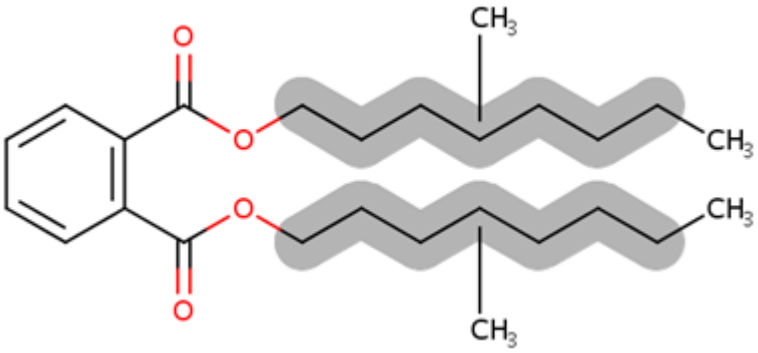

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ABBREVIATIONS AND ACRONYMS

AF	Assessment factor
bw	Body weight
COC	Concentration(s) of concern
dw	Dry weight
EC50	Effect concentration at which 50 percent of test organisms exhibit an effect
GD	Gestation day
HC05	Hazard concentration that is protective of 95 percent of the species in the sensitivity distribution
LC50	Lethal concentration at which 50 percent of test organisms die
LD50	Lethal dose at which 50 percent of test organisms die
LOAEL	Lowest-observed-adverse-effect level
LOEC	Lowest-observed-effect concentration
NITE	National Institute of Technology and Evaluation
NOAEL	No-observed-adverse-effect level
NOEC	No-observed-effect concentration
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPT	Office of Pollution Prevention and Toxics
PND	Postnatal day
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 evaluated the reasonably available information for environmental hazard endpoints associated with diisononyl phthalate (DINP) exposure. EPA reviewed 46 references and determined that 32 references had high or medium data quality. These references included acute and chronic exposures via water, soil, sediment, and food in both aquatic and terrestrial habitats.

Experimental aquatic hazard data were available from studies of the effects from acute exposures of DINP on five fish species, one amphibian species, five aquatic invertebrate species, and two algal species. Three fish taxa were represented in chronic exposure DINP feeding studies. Results from standard laboratory tests suggest that DINP has low hazard potential in aquatic species. Few adverse effects on survival, growth, development, or reproduction were observed in acute and chronic exposure duration tests at concentrations up to and exceeding the DINP solubility and saturation limits.

In terrestrial habitats, a Toxicity reference value (TRV) of 139 mg/kg-bw/d was derived for the chronic exposure effects of DINP on a generalized terrestrial mammal. One study of earthworm survival and reproduction found no hazards at the maximum experimental soil concentration of 1,000 mg/kg dw DINP. Also, no toxicity studies on avian or terrestrial plant species were identified.

1 INTRODUCTION

Diisononyl phthalate (DINP) is an organic substance primarily used as a plasticizer in a wide variety of consumer, commercial and industrial products (U.S. EPA, 2021b). Like most phthalates, DINP would be expected to cause adverse effects on aquatic organisms through a non-specific, narcotic mode of toxic action (Parkerton and Konkel, 2000); however, previous assessments have found few to no effects of DINP on organism survival and fitness (EC/HC, 2015; ECJRC, 2003). EPA reviewed studies of the potential toxicity of DINP to aquatic and terrestrial organisms and its potential environmental hazards.

2 APPROACH AND METHODOLOGY

EPA calculates hazard thresholds to identify potential concerns to aquatic and terrestrial species. For aquatic species, the hazard threshold is called a concentration of concern (COC), and for terrestrial species, the hazard threshold is called a hazard value or TRV. These terms (COC, TRV, and hazard value) describe how the values are derived and can encompass multiple taxa or ecologically relevant groups of taxa, as the environmental risk characterization serves populations of organisms within a wide diversity of environments. After weighing the scientific evidence, EPA selects the appropriate toxicity value from the integrated data to use for hazard thresholds. See Section 5 for more details about how EPA weighed the scientific evidence.

For terrestrial species, EPA estimates hazard by calculating a 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. When possible, EPA prefers to derive the TRV by calculating the geometric mean of the no-observed-adverse-effect-level (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 (according to methodology described in EPA's Guidance for Developing Ecological Screening levels (Eco-SSLs) (U.S. EPA, 2007), the TRVs for terrestrial mammals and birds are derived using a single endpoint.

During the scoping process, EPA reviewed the potential environmental hazards associated with DINP and identified 35 references (see Figure 2-9) from *Final Scope of the Risk Evaluation for Di-isononyl Phthalate (DINP) CASRN 28553-12-0 and 68515-48-0* (U.S. EPA, 2021c). EPA reviewed the environmental hazard data in these and additional referenced studies using the data quality evaluation metrics and criteria described in the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021a). Studies were assigned an overall quality determination of high, medium, low, or uninformative. High or medium data quality determinations were assigned to 19 aquatic organism references, several of which contained hazard data from multiple organisms and endpoints. EPA also considered 12 animal toxicity references that contained data used to determine a TRV, and 1 terrestrial earthworm toxicity reference. Thus, 32 references contained environmental hazard data with high or medium data quality determinations and were included in this assessment.

EPA assigned high or medium quality determinations to 19 aquatic toxicity references, and one terrestrial earthworm reference. Five references indicated hazard values from feeding or water-based exposure within fishes (Carnevali et al., 2019; Forner-Piquer et al., 2019; Forner-Piquer et al., 2018b; Forner-Piquer et al., 2018a; Patyna et al., 2006). All other studies did not result in estimates of population-level effects (*e.g.*, mortality, development, growth) up to the highest concentration tested. The maximum test concentrations reported in these aquatic studies exceeded the estimates of the water solubility limit for DINP which is approximately 6.1×10^{-4} mg/L (U.S. EPA, 2024). No studies on terrestrial wildlife vertebrate species (birds and mammals) were identified. In lieu of terrestrial wildlife studies, 12 references with controlled laboratory studies that used mice and rats as human health model organisms were used to calculate a TRV that is expressed as doses in units of mg/kg-bw/day. Although the TRV for DINP was derived from laboratory mice and rat studies, because body weight is normalized, EPA used it as a screening surrogate for effects on ecologically relevant wildlife species to evaluate chronic dietary exposure to DINP. An additional 12 studies of dietary DINP exposures to laboratory rodents with high or medium data quality evaluations were used to derive a TRV.

3 AQUATIC SPECIES HAZARD

EPA assigned an overall quality level of high or medium to 19 references. These references contained relevant aquatic toxicity data for sheepshead minnow (*Cyprinodon variegatus*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis macrochirus*), Japanese medaka (*Oryzias latipes*), gilthead sea bream (*Sparus aurata*), moorfrog (*Rana arvalis*), waterflea *(Daphnia magna),* amphipod (*Hyalella azteca*), midge (*Paratanytarsus parthenogenetica* & *Chironomus tentans*), mysid shrimp (*Americamysis bahia*), green algae (*Selanastrum capricornutum*), and marine dinoflagellate (*Karinia brevis*). EPA summarized aquatic toxicity studies for quantitative assessment of aquatic vertebrates (Table 3-1), invertebrates (Table 3-2), and algae (Table 3-3).

Aquatic Vertebrates

Fish: EPA identified references with data from acute exposures and chronic exposures of DINP on different fishes. Acute exposure studies found no effects of DINP at any of the tested concentrations (Table 3-1). Chronic studies found no effects of DINP water exposure on fish and found inconsistent effects of dietary exposure to fish.

Seven of the eight acute studies on aquatic vertebrates consisted of 96-hour toxicity tests conducted on juvenile and adult fish species and were all assigned overall quality determinations of high. These acute exposure studies tested up to the limit of solubility, were conducted without the use of solvents, and were not able to establish LC50 or LOEC values due to lack of mortality. Also, the maximum test concentrations reported in these studies exceeded EPA's estimate of the water solubility limit for DINP which is approximately 6.1×10^{-4} mg/L (U.S. EPA, 2024). One study with an assigned overall quality determination of medium used 0.1 percent methanol as a solvent to enhance solubility and reported an LC50 of greater than 500 mg/L (the highest tested nominal concentration) from 72-hour exposures with newly fertilized zebrafish embryos (4–128 cell stage) (Chen et al., 2014).

Of the studies of chronic dietary DINP exposure, a chronic duration study with Japanese medaka (*Oryzias latipes*) with a high data quality determination found statistically significant but inconsistent effects of DINP-amended diets on survival in second-generation fish, but not first- or third-generation fish (Patyna et al., 2006). This two-generation feeding study fed one elevated dose of 1 mg/kg-bw/day DINP-amended dried food to juvenile and adult fish. Lower survival of embryos occurred in one assay of F_0 embryos, but not during a second assay in the F_0 generation or in multiple assays in the F_1 and F_2 generations. Thus, fish embryos exhibited an inconsistent effect of parental dietary exposure to 1 mg/kg-bw/day DINP with most assays finding no effects across three generations. The study also found a transient effect of 16 percent lower survival among F_1 adult fish fed 1 mg/g-bw/day DINP over 140 days compared to control fish. This effect on survival did not occur in the F_0 generation despite identical dietary exposure over 140 days or in the F_2 generation despite 40 more days of dietary exposure. Thus, dietary DINP induced a transient 16 percent reduction in survival only in the second generation of continuous feeding exposure, but not in the first or third generations. The authors measured several other endpoints and found no DINP effects on reproduction and development except for an increase in testosterone metabolites in males and a delay in red blood cell pigmentation in fish fed DINP daily. The DINP-amended food dose was analytically verified as 21.9 ± 2.8 µg/g fed at a rate of 5 percent body weight per day with brine shrimp fed as a supplement three times per week for the F_0 generation and five times per week for the F_1 generation resulting in an average lipid-based feeding rate of 1 mg/kg-bw/day DINP per fish. Patyna et al. (2006) conducted this study with five replicates per treatment and included untreated and solvent (acetone) controls.

Three 21-day feeding studies on gilthead sea bream (*S. aurata*) with overall quality determinations of

medium, found non-apical effects of DINP on fish (Carnevali et al., 2019 and Forner-Piquer,2019, 5534689 and Forner-Piquer,2019, 5534689; Forner-Piquer et al., 2018a). A 21-day study of gilthead sea bream fed with 1.5 mg/kg-bw DINP-amended food resulted in increased presence of lipids and triglycerides and decreased glycogen and phospholipids in the liver (Forner-Piquer et al., 2018a). This study also found DINP exposure upregulated genes associated with disrupted metabolic activity. No statistically significant differences in body mass were observed among treatments, however. Similarly, Carnevali et al. (2019) found that gilthead sea bream exhibited decreased muscle protein and lipid content after being fed 1.5 mg/kg-bw DINP, which was the highest nominal concentration of DINP administered. This study also found that dietary DINP exposure resulted in upregulated *catd* mRNA levels and more enzymes that break down proteins. Finally, Forner-Piquer et al. (2019) found reduced levels of endocannabinoids and endocannabinoid-like mediators along with higher fatty acid amide hydrolase activity in gilthead sea bream fed 1.5 mg/kg bw DINP per day compared to no-DINP controls and low-DINP treatments of 15 µg/kg-bw DINP per day. The authors documented fewer motile sperm cells due to DINP despite overall sperm production being unaffected. The production of 11-ketotestosterone, which is an active androgen in fish, was greater than 50 percent lower in males fed diets of 1.5 mg/kg-bw DINP per day compared to no-DINP control fish after 21 days. EPA has slight confidence in the hazard values from all three of these studies for several reasons that they all share.

First, all effects were non-apical in that they were not directly related to fish survival, growth, or reproduction. Second, each study used experimental designs and analyses that resulted in a mismatch between experimental unit replication and the statistical and biological inferences that were made. For example, treatment diets were given to fish in duplicate (*i.e.*, five fish in each of two aquaria, or n = 2), but results were presented from analyses using individual fish as replicates (*i.e.*, n = 10). Thus, inferences about the results could be inferred about the tissues of individual fish but not a population of fish. Third, the studies did not analytically verify DINP concentrations in the food and relied on nominal concentrations across 21 days. Finally, the relatively short duration (21-day) of feeding exposure to adult fish may be inadequate for detecting apical effects that are most likely to translate to effects on fish populations.

In a chronic 21-day DINP adult zebrafish study with a data quality determination of high, Santangeli et al. (2017) reported 30 percent reductions in eggs per female and gonadosomatic index at a water concentration of 0.42 µg/L DINP compared to controls. However, the effects disappeared at higher nominal DINP concentrations of 4.2, 42, 420, and 4,200 µg/L. The nominal concentrations of 0.42, 4.2, 42, 420, and 4,200 µg/L were not analytically verified and exceeded water solubility. Also, the study was conducted with treatment and control groups in duplicate with all fish in each treatment concentration housed in a single net-divided aquarium, resulting in limited statistical power. Although this study received an initial high data quality determination, EPA has low confidence in the reported effects due to the lack of dose-response effects, analytical DINP verification, and experimental unit replication.

An additional chronic study, with an overall quality determination of medium, exposed zebrafish to multiple water concentrations of DINP (Forner-Piquer et al., 2018b). Forner-Piquer et al. (2018b) found >30 percent reductions in zebrafish fertilization rates, greater than 20 percent reductions in gonadosomatic index, and statistically significant changes in a number of lipid-signaling endpoints at the lowest exposure concentration of 0.42 µg/L DINP over 21 days. However, these effects were not observed at higher nominal DINP concentrations of 4.2 and 42 µg/L. These concentrations were not analytically verified, exceeded water solubility, and exposure was only replicated twice resulting in limited statistical power.

EPA identified one study on an amphibian, the moorfrog (*R. arvalis*), and assigned an overall quality determination of high (IVL, 2001). Moorfrog embryos were exposed to two sediment types (fine and coarse) spiked with nominal DINP concentrations of 0 (negative control), 0 (acetone solvent control), 100, 300, and 1,000 mg DINP/kg-dw to investigate hatchability and embryo survival with observations at 9, 12, 16, and 21 days of exposure. Hatching success, median hatching time, mortality, and deformities were not statistically different among DINP, control and solvent control treatments. Tadpole growth, recorded as wet weight, was assessed after 26 days of exposure with results indicating no difference among DINP, control, and solvent control treatments.

296 **Table 3-1. Aquatic Vertebrate Environmental Hazard Studies for DINP**

Duration	Test Organism (Species)	Endpoint	Hazard Values	Effect	Citation (Data Evaluation Rating)
Acute	Sheepshead minnow (*Cyprinodon variegatus*)	96-hour LC50	>0.52 mg/L [a]	Mortality	(Adams et al., 1995) (High)
	Rainbow trout (*Oncorhynchus mykiss*)	96-hour LC50	>0.16 mg/L [a]	Mortality	(Adams et al., 1995) (High)
	Fathead minnow (*Pimephales promelas*)	96-hour LC50 (static)	>0.10 mg/L [a]	Mortality	(Adams et al., 1995) (High)
	Fathead minnow (*Pimephales promelas*)	96-hour LC50	>0.14 mg/L [a]	Mortality	(EG & G Bionomics, 1983a) (High)
	Fathead minnow (*Pimephales promelas*)	96-hour LC50 (flow-through)	>0.19 mg/L [a]	Mortality	(Adams et al., 1995) (High)
	Bluegill sunfish (*Lepomis macrochirus*)	96-hour LC50	>0.14 mg/L [a]	Mortality	(Adams et al., 1995) (High)
	Bluegill sunfish (*Lepomis macrochirus*)	96-hour LC50	>0.17 mg/L [a]	Mortality	(EG & G Bionomics, 1983c) (High)
	Zebrafish (*Danio rerio*)	72-hour LC50	>500 mg/L [b]	Mortality	(Chen et al., 2014) (Medium)
Chronic	Japanese Medaka (*Oryzias latipes*)	2nd generation 140-day LOEC	1 mg/kg bw/day [a c]	Posthatch survival	(Patyna et al., 2006) (High)
	Japanese Medaka (*Oryzias latipes*)	2nd generation 140-day LOEC	1 mg/kg bw/day [a d]	Survival/Reproduction /Growth	(Patyna et al., 2006) (High)
	Zebrafish (*Danio rerio*)	21-day LOEC	>0.0004 mg/L [a]	Egg production, oocyte biochemical composition	(Santangeli et al., 2017) (High)
	Zebrafish (*Danio rerio*)	21-day LOEC	0.0004 mg/L [a]	Egg production, lipid signaling system	(Forner-Piquer et al., 2018b) (Medium)
	Gilthead sea bream (*Sparus aurata*)	21-day LOEC	1.5 mg/kg bw [b e]	Muscle molecular composition	(Carnevali et al., 2019) (Medium)
	Gilthead sea bream (*Sparus aurata*)	21-day LOEC	1.5 mg/kg bw [b f]	Lipid signaling system	(Forner-Piquer et al., 2018a) (Medium)
	Gilthead sea bream (*Sparus aurata*)	21-day LOEC	1.5 mg/kg bw [b g]	Increase in Gonadosomatic Index	(Forner-Piquer et al., 2019) (Medium)
	Moorfrog (*Rana arvalis*)	21-day LOEC	>1,000 mg/kg/ dry weight [a]	Hatching success, mortality, growth	(IVL, 2001) (High)

Duration	Test Organism (Species)	Endpoint	Hazard Values	Effect	Citation (Data Evaluation Rating)
[a] indicates measured concentration. [b] indicates nominal concentration. [c] authors state that posthatch survival was lower in one assay of the F_0 generation, but not in a second assay of the F_0 generation and no posthatch survival effects were observed in the F_1 or F_2 embryos. DINP diets delayed the pigmentation of red blood cells and increased testosterone hydroxylase activity. [d] dietary DINP induced a transient 16% reduction in survival after 140 days of exposure to parental and then another 140 days of individual fish exposure. DINP effects were not observed in F_0 or F_2 generations. The authors concluded that DINP in diet did not affect adult fish survival overall. [e] dietary DINP exposure resulted in decreased lipid and protein content in muscle tissue due to upregulated catd mRNA levels and more enzymes that break down proteins. [f] dietary DINP exposure resulted in more lipids and triglycerides in fish livers along with upregulated genes associated with disrupted metabolic activity. [g] dietary DINP exposure resulted in lipid metabolism disruption, reduced androgen production, increase 17β-estradiol production leading to a higher gonadosomatic index, and fewer motile sperm cells in male fish.					

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Aquatic Invertebrates

EPA identified 11 studies with aquatic invertebrate hazard data from DINP exposure: seven studies representing acute DINP exposures and four studies representing chronic DINP exposures, all with overall quality determinations of high (Table 3-2).

Acute studies conducted on aquatic invertebrates included results of three different 48-hour exposures of DINP to *D. magna*, one 48-hour exposure and one 96-hour exposure of DINP to *P. parthenogenetica*, and two different 96-hour exposures of DINP to *A. bahia*, (Brown et al., 1998; Adams et al., 1995; EG & G Bionomics, 1984b; Springborn Bionomics, 1984a). Adverse acute effects were not observed at DINP concentrations up to and beyond the limit of solubility (6.1×10^{-4}, (U.S. EPA, 2024). For example, Springborn Bionomics (1984a) studied the acute toxicity of 14 phthalate esters to *D. magna* under static conditions. Based on the 0 and 48 hour mean measured concentrations, the DINP EC50 exceeded 0.086 mg/L. No visible film or apparent insoluble test material was observed in the test solution; however, since there were entrapped daphnids on the test vessel's surface, the authors suggested that the test material aggregated on the surface during tests. No mortality was reported even though more than 50 percent of the daphnids were caught on the surface of the test solution.

Chronic studies with aquatic invertebrates included two aquatic DINP exposures on *D. magna* (Brown et al., 1998; Rhodes et al., 1995) and two 10-day studies with sediment DINP exposures conducted on *H. azteca* and *C. tentans* (Call et al., 2001). *D. magna* exposed to nominal concentrations of DINP for 21 days resulted in a reduced survival and reproduction LOEC of 0.089 mg/L and a NOEC of 0.034 mg/L, for a chronic value (ChV) of 0.06 mg/L (Rhodes et al., 1995). Although authors reported that no visible film was observed, physical entrapment of *D. magna* with the water surface boundary was observed within test vessels at the LOEC. The authors concluded that this physical entrapment contributed to their observed animal mortality and reproduction effects. Thus, because of this uncertainly between physical and chemical toxicity, EPA is not considering these as concentrations of concern. A similar 21-day exposure study conducted by Brown et al. (1998) increased the solubility of DINP in solution with the addition of a dispersant, castor oil 40 ethoxylate (10 mg/L) and found no differences in reproduction or survival from a 1 mg/L exposure to DINP when compared to the control or dispersant control. Longer duration studies with *C. tentans* and *H. azteca* were conducted with subchronic 10-day exposures of sediment spiked with nominal concentrations of DINP (Call et al., 2001). Adverse effects were not observed for the highest DINP spiked sediment concentrations used in these studies at 2,900 mg/kg dw DINP sediment and 2,680 mg/kg dw DINP sediment for *H. azteca* and *C. tentans*, respectively.

Table 3-2. Aquatic Invertebrate Environmental Hazard Studies for DINP

Duration	Test Organism (Species)	Endpoint	Hazard Values[a]	Effect	Citation (Data Evaluation Rating)
Acute	Waterflea (*Daphnia magna)*	48-hour EC50	>0.06 mg/L	Immobilization	(Adams et al., 1995) (High)
	Waterflea (*Daphnia magna)*	48-hour EC50	>1.00 mg/L	Immobilization	(Brown et al., 1998) (High)
	Waterflea (*Daphnia magna)*	48-hour EC50	>0.09 mg/L	Immobilization	(Springborn Bionomics, 1984a) (Medium)
	Midge (*Paratanytarsus parthenogenetica*)	48-hour LC50	>0.12 mg/L	Mortality	(EG & G Bionomics, 1984c) (High)
	Midge (*Paratanytarsus parthenogenetica*)	96-hour LC50	>0.08 mg/L	Mortality	(Adams et al., 1995) (High)
	Mysid shrimp (*Americamysis bahia*)	96-hour LC50	>0.39 mg/L	Mortality	(Adams et al., 1995) (High)
	Mysid shrimp (*Americamysis bahia*)	96-hour LC50	>0.77 mg/L	Mortality	(EG & G Bionomics, 1984b) (High)
Chronic	Waterflea (*Daphnia magna*)	21-day LOEC	0.034 mg/L NOEC 0.089 mg/L LOEC for all effects [b]	Mortality, Offspring per female	(Rhodes et al., 1995) (High)
	Waterflea (*Daphnia magna*)	21-day NOEC	>1.0 mg/L	Mortality, Reproduction, Growth	(Brown et al., 1998) (High)
	Amphipod (*Hyalella azteca*)	10-day NOEC	>0.44 mg/L porewater; >2900 mg/kg dw sediment	Mortality	(Call et al., 2001) (High)
	Midge (*Chironomus tentans*)	10-day NOEC	>0.869 mg/L porewater, >2680 mg/kg dw sediment	Mortality	(Call et al., 2001) (High)

[a] all hazard values represent measured concentrations.
[b] the authors concluded that *D. magna* physical entrapment with surface tension contributed to animal mortality and reproduction effects.

Aquatic Plants

EPA identified two studies with an overall quality determination of high and one study with an overall quality determination of low for aquatic plants exposed to DINP (Table 3-3).

Both studies with overall quality determinations of high were conducted on green algae, *Salanastrum capricornutum*. Springborn Bionomics (1984c) determined that an EC50 based on cell numbers at 5 days of DINP exposure was greater than2.8 mg/L, well over EPA's estimated water solubility of 6.1×10^{-4} (U.S. EPA, 2024). Specifically, chlorophyll *a* concentration was not different from the control treatment after 5 days of DINP exposure but cell numbers within the single DINP concentration tested (2.8 mg/L) were 34 percent less than the control treatment. Adams et al. (1995) did not observe adverse effects at the highest tested concentration of DINP (1.8 mg/L) from 96-hour exposures of DINP. Concentrations of DINP were verified analytically with gas-liquid chromatography and gas chromatography for Springborn Bionomics (1984c) and Adams et al. (1995), respectively, with neither study using a solvent within treatment and control groups. A 96-hour exposure study conducted on the marine dinoflagellate, *K. brevis*, resulted in no significant effect of DINP on algal cell number compared to the controls up to the highest reported nominal concentration of DINP at 50 mg/L (Liu et al., 2016).

Table 3-3. Aquatic Plant Environmental Hazard Studies for DINP

Test Organism (Species)	Endpoint	Hazard Values	Effect	Citation (Data Evaluation Rating)
Green algae (*Selanastrum capricornutum*)	96-hour EC50	>2.80 mg/L [a]	Cell numbers, chlorophyll *a*	(Springborn Bionomics, 1984c) (High)
Green algae (*Selanastrum capricornutum*)	96-hour EC50	>1.80 mg/L [a]	Cell numbers	(Adams et al., 1995) (High)
Marine dinoflagellate (*Karinia brevis*)	96-hour EC50	>50 mg/L [b]	Cell numbers	(Liu et al., 2016) (Low)

[a] indicates measured concentration.
[b] indicates nominal concentration.

3.1 Aquatic Organism Hazard Conclusions

Overall, EPA has robust confidence in the evidence that DINP has low hazard potential in aquatic species (Table 5-1). No consistent effects of DINP on aquatic organism survival or reproduction were observed in studies of aquatic organisms across taxonomic groups, habitats, exposure type, and exposure duration. Studies of DINP exposure via water to fish, amphibians, invertebrates, and algae reported no effects up to and well above the solubility limit in the water column and in the sediment pore water. Studies of dietary exposure of DINP to two fish species indicate no consistent population-level DINP effects and inconsistent effects of DINP on mechanistic endpoints such as gene expression and protein synthesis. Thus, EPA has moderate confidence in the studies that describe the potential effects of chronic dietary DINP exposure to fish populations.

4 TERRESTRIAL SPECIES HAZARD

EPA identified 12 terrestrial animal toxicity references with overall quality determinations of high or medium that use rat (*Rattus norvegicus*) or mouse (*Mus musculus*) species to study reproductive, growth, or survival endpoints. These studies were used to derive a TRV of DINP for a representative small mammal. EPA also identified one invertebrate toxicity study on chronic exposure of DINP to earthworms (*Eisenia fetida*) in soil.

Terrestrial Vertebrates

No terrestrial vertebrate studies were reasonably available to assess the potential effects or hazards from DINP exposure in bird or mammalian wildlife species. Therefore, EPA considered ecologically relevant definitive hazard data from studies conducted on laboratory mammals (*e.g.*, rats, mice, etc.) that are routinely used to inform human health hazard. These data were then used in accordance with EPA's Guidance for Developing Ecological Soil Screening Levels (Eco-SSLs) (U.S. EPA, 2007) to formulate a TRV to represent terrestrial mammals (see Table 4-1 and Table 6-1).

Mammals

Multiple studies of DINP administered in rat diets found reductions in rat offspring body weight over the course of 2 to 19 weeks (LOAEL range from 288 to 1,500 mg/kg-bw/d) (Gray, 2023; Clewell et al., 2013; Boberg et al., 2011; Masutomi et al., 2003; NTP-CERHR, 2003; Waterman et al., 2000). Masutomi et al. (2003) found a decrease in body weight of male pups at prepubertal necropsy (PND 27) in 306.7 and 1,165.5 mg/kg/day groups. Exposure duration was 18 days (assuming GD 15–22, PND 1–10) and ceased on PND 10. Dams were fed control diet for the remainder of lactation, and pups were fed the control diet after weaning. Treatment exposures were 0, 30.7, 306.7 and 1,164.5 mg/kg/day). Hellwig et al. (1997) found mean maternal body weights were lower in rats gavaged 1,000 mg/kg DINP at days 13, 15, and 17 post gestation day after being administered DINP from day 6 to day 15 post gestation day. Waterman et al. (1999) found reductions in maternal rat body weight gain in 1,000 mg/kg/day treatments after being gavaged from GD 6 to 15. In a one-generation study, Exxon Biomedical (1996a) found lower body weight in parental female rats in 741 mg/kg/day and 1,087 mg/kg/day groups during GD 0 to 21. Rats were fed DINP in diet through gestation and post-partum.

In similar two-generation studies, Exxon Biomedical (1996b) found lower body weight of F_1 male pups and F_1 female pups at birth (PND 0) in the 0.4 and 0.8 percent dietary concentrations groups and lower body weight as GD 21 of P1 adult females. Boberg et al. (2011) found lower male pup weight at PND 13 in a 900 mg/kg bw/day DINP-fed treatment. Exposure duration was 33 days (GD 7–22, PND 1–17). Finally, Clewell et al. (2013) found lower male pup weight on PND 14 at 247 mg/kg/day DINP treatments. Adult rats were fed DINP diets through gestation and lactation.

Growth: Across a range of study durations, DINP fed to adult rats resulted in lighter body weights compared to control adult rats and mice (LOAEL range 152 to 1,513 mg/kg/d) (Clewell et al., 2013; Masutomi et al., 2003; NTP-CERHR, 2003; Waterman et al., 2000; Covance Labs, 1998c; Lington et al., 1997; Bio/dynamics, 1987). Bio/dynamics (1987) found lower body weight in high dose (672 mg/kg-bw/day) females during most timepoints from week 11 through 94. In a 104-week feeding study with mice, Covance Labs (1998c) found lower body weight in 741 mg/kg-bw/day DINP diet fed male mice. This effect was consistent in weeks 29 through 105. The same study found a lower female mouse body weights when fed 741 mg/kg-bw/day DINP. This effect was observed in weeks 29, 37, and weeks 45 through 105. In one- and two-generation studies with rats, Exxon Biomedical (1996a) found reductions in parental male and female body weights in both generations at feeding doses as low as 301 mg/kg-bw/day in the first generation and 288 mg/kg-bw/day DINP in the second generation.

Survival: In studies of adult rat survival, fewer rats survived while being fed DINP compared to control rats (LOAEL range 184 to 733.2 mg/kg/d) (Covance Labs, 1998c; Lington et al., 1997). DINP diets also lowered the survival of adult mice compared to controls (LOAEL=1560.2 mg/kg/d) (Covance Labs, 1998a; Lington et al., 1997). Using these studies and guidance from Eco-SSLs (U.S. EPA, 2007),

Avian

No avian hazard studies were reasonably available to assess potential hazards from DINP exposure.

Table 4-1. Terrestrial Mammal Hazard Studies of DINP Used for TRV Derivation

Test Organism	NOAEL/ LOAEL (mg/kg-day)	Effect	Study Description (Duration/Dose)	Citation (Rating)
Sprague-Dawley rat (*Rattus norvegicus*)	31/307	Reproduction: lower male pup body weight at prepubertal necropsy (PND 27)	18-day diet exposure to maternal females (GD 15–22, PND 1–10). Target concentrations were 400, 4,000, and 20,000 ppm (0, 30.7, 306.7 and 1,164.5 mg/kg/day).	(Masutomi et al., 2003) (Medium)
Wistar rat (*Rattus norvegicus*)	200/1000	Reproduction: lower maternal body weights in the 1,000 mg/kg group at GD 15	10-day gavage exposure to pregnant females (GD 6–15). Target concentrations were 0, 40, 200, 1,000 mg/kg/day.	(Hellwig et al., 1997) (Medium)
Sprague-Dawley rat (*Rattus norvegicus*)	500/1000	Reproduction: lower maternal body weight gain GD 6–9 and 6–15.	10-day gavage exposure to pregnant females (GD 6–15). Target concentrations were 0, 100, 500, 1,000 mg/kg/day.	(Waterman et al., 1999) (High)
Sprague-Dawley rat (*Rattus norvegicus*)	377/741	Reproduction: lower maternal body weight at GD 21	One generation study diet exposure (10 weeks prior to mating, through mating, gestation, and lactation). Target doses correspond to dietary concentrations of 0, 0.5, 1, and 1.5% (0, 377, 741, 1,087 mg/kg/day).	(Exxon Biomedical, 1996a) (Medium)
Sprague-Dawley rat (*Rattus norvegicus*)	287/555	Reproduction: lower male F1 body weight at birth (PND 0)	Two-generation study diet exposure. Target doses correspond to dietary concentrations of 0, 0.2, 0.4, and 0.8% (0, 146, 287, 555 mg/kg/day).	(Exxon Biomedical, 1996b) (High)
Sprague-Dawley rat (*Rattus norvegicus*)	139/274	Reproduction: lower male F2 offspring body weight at PND 7 and PND 21.	Two-generation study diet exposure. Target doses correspond to dietary concentrations of 0, 0.2, 0.4, and 0.8% (0, 143, 288, 560 mg/kg/day).	(Exxon Biomedical, 1996b) (High)
Wistar rat (*Rattus norvegicus*)	750/900	Reproduction: lower male pup weight at PND 13	33-day gavage exposure to maternal females (GD 7–22, PND 1–17). Target concentrations were 0, 300, 600, 750, 900 mg/kg/day.	(Boberg et al., 2011) (Medium)

Test Organism	NOAEL/ LOAEL (mg/kg-day)	Effect	Study Description (Duration/Dose)	Citation (Rating)
Sprague-Dawley rat (*Rattus norvegicus*)	56/288	Reproduction: lower male pup body weight at PND 14	25-day feeding exposure to gestational and lactating females (GD 12 through PND 14). Target doses correspond to dietary concentrations of 0, 760, 3,800, and 11,400 ppm (0, 56, 288, 720 mg/kg/day).	(Clewell et al., 2013) (Medium)
Sprague-Dawley rat (*Rattus norvegicus*)	307/1165	Growth: lower maternal body weight (PND 2–10)	18-day dietary exposure to maternal animals (GD 15 to PND 10). Target concentrations were 0, 400, 4,000, and 20,000 ppm (30.7, 306.7 and 1,164.5 mg/kg/day).	(Masutomi et al., 2003) (Medium)
Sprague-Dawley rat (*Rattus norvegicus*)	331/672	Growth: lower maternal body weight (PND 2–10)	2-year chronic dietary. Doses correspond to dietary concentrations of 0, 33, 331, and 672 mg/kg/day.	(Bio/dynamics, 1987) (High)
Fischer 344 rat (*Rattus norvegicus)*	88/359	Growth: lower male body weight gain	Chronic (105-week) diet exposure. Target dietary doses were 0, 500, 1,500, 6,000, and 12,000 ppm (0, 29.2, 88.3, 358.7, 733.2 mg/kg/day).	(Covance Labs, 1998c) (High)
Sprague-Dawley rat (*Rattus norvegicus*)	301/622	Growth: lower male body weight	One-generation reproduction study. Target dietary concentrations were 0, 0.5, 1, and 1.5% (0, 301, 622, 966 mg/kg/day).	(Exxon Biomedical, 1996a) (Medium)
Sprague-Dawley rat (*Rattus norvegicus*)	363/734	Growth: lower parental female body weight	One-generation reproduction study. Target dietary concentrations were 0, 0.5, 1, and 1.5% (0, 363, 734, 1,114 mg/kg/day).	(Exxon Biomedical, 1996a) (Medium)
Sprague-Dawley rat (*Rattus norvegicus*)	347/673	Growth: lower P1 adult body weight	Two-generation reproduction study. Target dietary concentrations were 0, 0.2, 0.4, and 0.8% (0, 146, 287, 555 mg/kg/day).	(Exxon Biomedical, 1996b) (High)
Sprague-Dawley rat (*Rattus norvegicus*)	348/718	Growth: lower P2 adult female body weight	Two-generation reproduction study. Target dietary concentrations were 0, 0.2, 0.4, and 0.8% (0, 143, 288, 560 mg/kg/day).	(Exxon Biomedical, 1996b) (Medium)
B6C3F1 Mouse (*Mus musculus*)	276/742	Growth: lower adult male body weight	104-week dietary exposure to adult mice. Target dietary concentrations were 0, 500, 1,500, 4,000, and 8,000 ppm (0, 90.3, 275.6, 741.8, 1,560.2 mg/kg/day).	(Covance Labs, 1998b) (High)
B6C3F1 Mouse (*Mus musculus*)	336/910	Growth: lower adult female body weight	104-week exposure to adult mice. Target dietary concentrations were 0, 500, 1,500, 4,000, and 8,000 ppm (0, 112, 335.6, 910.3, 1,887.6 mg/kg/day).	(Covance Labs, 1998b) (High)

Test Organism	NOAEL/ LOAEL (mg/kg-day)	Effect	Study Description (Duration/Dose)	Citation (Rating)
Fischer 344 rat (*Rattus norvegicus*)	15/152	Growth: lower adult male body weight	Chronic (2-year) dietary study in rats. Target dietary concentrations were 0, 0.03, 0.3, and 0.6% (0, 15, 152, 307 mg/kg/day).	(Bio/dynamics, 1986) (High)
Sprague-Dawley rat (*Rattus norvegicus*)	555/1513	Growth: lower maternal body weight at PND 2 and PND 14	25-day exposure (GD 12 through PND 14) to maternal rats. Target dietary concentrations were 0, 760, 3,800, and 11,400 ppm (0, 109, 555, 1513 mg/kg/day).	(Clewell et al., 2013) (Medium)
Fischer 344 rat (*Rattus norvegicus*)	1192/2289	Growth: lower male body weight	21-day dietary exposure to male and female rats. Target dietary concentrations were 0, 0.6, 1.2, and 2.5% (0, 639, 1,192, 2,195 mg/kg/day).	(Barber et al., 1987) (Medium)
Fischer 344 rat (*Rattus norvegicus*)	359/733	Survival: lower male survival	105-week dietary exposure to male and female rats. Target dietary concentrations were 0, 500, 1,500, 6,000, and 12,000 ppm (0, 29.2, 88.3, 358.7, 733.2 mg/kg/day).	(Covance Labs, 1998c) (High)
B6C3F1 Mouse (*Mus musculus*)	742/1560	Survival: lower male survival	104-week dietary exposure to male and female mice. Target dietary concentrations were 0, 500, 1,500, 4,000, and 8,000 ppm (0, 90.3, 275.6, 741.8, 1,560.2 mg/kg/day).	(Covance Labs, 1998b) (High)
Fischer 344 rat (*Rattus norvegicus*)	18/184	Survival: lower female survival	2-year dietary exposure to male and female rats. Target dietary concentrations were 0, 0.03, 0.3, and 0.6% (0, 18, 184, 375 mg/kg/day).	(Bio/dynamics, 1986) (High)

Terrestrial Invertebrates

EPA identified one study of DINP chronic exposure to the earthworm *Eisenia fetida* in artificial soil (ExxonMobil, 2010). This study found no difference in mortality between earthworms in control soil and soil containing nominal concentrations of 1,000 mg/kg dw DINP. The soil concentrations were analyzed by gas chromatography with flame ionization detection and ranged from 925.2 to 1052 mg/kg on Day 0 and from 651.4 to 795.8 mg/kg on Day 28 and from 389.6 to477.1 mg/kg on Day 56. However, the study found a difference between the number of juveniles found in 1,000 mg/kg dw DINP soils (mean=90) versus a mean of 39 worms found in no-DINP control soils.

Terrestrial Plants

No terrestrial plants studies were available to assess potential hazards from DINP exposure.

4.1 Terrestrial Organism Hazard Conclusions

Overall, EPA has moderate confidence in the evidence that DINP poses low hazard to terrestrial mammals via dietary exposure, but robust confidence that DINP poses no hazard to soil invertebrates (see Table 5-1). No studies on DINP exposure to wild mammals, birds, or plants were available to assess DINP hazard, indicating that no hazard has been observed in these groups under realistic exposure

conditions. EPA reviewed studies of laboratory rodents to derive a TRV of 139 mg/kg-bw/day dietary DINP exposure. This TRV represents the potential chronic exposure dose at which the dietary effects of DINP may affect a general mammal. Thus, EPA has only moderate confidence that the TRV represents realistic hazards to wild populations. Chronic DINP exposure to an earthworm species in soil did not affect earthworm survival, indicating little to no hazard of DINP to soil dwelling invertebrates.

5 WEIGHT OF SCIENTIFIC EVIDENCE CONCLUSIONS FOR ENVIRONMENTAL HAZARD

Overall, EPA has determined that DINP poses low hazard potential in aquatic species and has robust confidence in the evidence showing low acute aquatic hazard, low acute benthic hazard, low chronic benthic hazard, and low aquatic plant hazard and moderate confidence in the evidence showing low chronic aquatic hazard to fish (see Aquatic Organism Hazard Conclusions). Within the terrestrial environment, EPA has moderate confidence in the evidence showing low chronic dietary hazards of DINP to terrestrial mammals and robust confidence in the evidence for low soil invertebrate hazard (see Terrestrial Organism Hazard Conclusions). Thus, the weight of scientific evidence leads EPA to having robust confidence in the overall conclusion that DINP has little to no hazards to wild organism populations. However, EPA has more uncertainty and less confidence in the size and quality of the studies in the database, the strength and precision of more subtle and mechanistic effects found within a few studies, and whether study design allowed for dose-response effects to be detected for mechanistic endpoints. A more detailed explanation of the weight of the scientific evidence, uncertainties, and overall confidence levels is presented in Appendix A.1. EPA uses several considerations when weighing the scientific evidence to determine confidence in the environmental hazard data. These considerations include the quality of the database, consistency, strength and precision, biological gradient/dose response, and relevance (see Appendix A.2), and are consistent with the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021a). Table 5-1 summarizes how these considerations were determined for each environmental hazard.

Table 5-1. DINP 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[a]	Hazard Confidence[b]
Aquatic						
Acute aquatic assessment	+++	+++	+++	++	+++	Robust
Acute benthic assessment	++	+++	+++	++	+	Robust
Chronic aquatic assessment	++	+	+	+	+++	Moderate
Chronic benthic assessment	++	++	++	+	+++	Robust
Algal assessment	+	+++	++	++	+++	Robust
Terrestrial						
Avian assessment	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate
Chronic mammalian assessment	++	+++	+++	+++	+	Moderate
Soil invertebrate assessment	+	Not applicable	+	+	+++	Robust
Terrestrial plant assessment	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate

[a] Relevance includes biological, physical/chemical (including use of analogues), and environmental relevance.
[b] Hazard Confidence reflects the overall confidence in the conclusions about the presence or absence of hazard thresholds and the weight of support and uncertainties around all the available data and does not necessarily represent a summation of the individual evidence properties.
+++ 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

Aquatic Species Hazard Values

Acute Aquatic Threshold: No definitive hazard values or concentrations of concern were identified from the studies of acute exposure of DINP on aquatic organisms that live in the water column. Thus, EPA found no hazards from acute water exposure of DINP to aquatic organisms.

Acute Benthic Threshold: No definitive hazard values or concentrations of concern were identified from the studies of acute exposure of DINP on benthic organisms. Thus, EPA found no hazards from acute exposure of DINP to aquatic organisms living in benthic habitats.

Chronic Aquatic Threshold: No definitive hazard concentrations via water or dietary exposure were identified from the studies of chronic exposure of DINP on aquatic organisms. Thus, EPA found no survival or reproductive hazards of chronic DINP to aquatic organism populations.

Chronic Benthic Threshold: No definitive hazard values or concentrations of concern were identified from the studies of chronic exposure of DINP on benthic organisms. Thus, EPA found no hazards from chronic exposure of DINP to aquatic organisms living in benthic habitats.

Aquatic Plant Threshold: No definitive hazard values or concentrations of concern were identified from the studies of DINP effects on algae. Thus, EPA found no hazards from acute or chronic exposure of DINP to aquatic plants.

Terrestrial Species Hazard Values

Terrestrial Vertebrate Threshold: For terrestrial species exposed to DINP, EPA estimated hazard using a deterministic approach to calculate a TRV expressed as doses in units of mg/kg-bw/day (for mammals) (Figure 6-1). Although the TRV for DINP was derived from laboratory mice and rat studies, body weight was standardized, therefore the TRV can be used with ecologically relevant wildlife species to evaluate the potential toxicity of chronic dietary exposure to DINP. The following criteria and steps (Figure 6-2) were used to select the data to calculate the TRV for DINP with NOAEL and/or LOAEL data using (U.S. EPA, 2007). General step descriptions are in italics, while EPA's step by step decisions for DINP are in regular text (Figure 6-2).

Step 1: The minimum data set required to derive either a mammalian or avian TRV consists of three results (NOAEL or LOAEL values) for reproduction, growth, or mortality for at least two mammalian or avian species.

EPA assessed 12 studies with 24 reported NOAELs and 24 reported LOAELs. The studies included multiple strains of rat (*R. norvegicus*) including Sprague-Dawley, Wistar, and Fischer344, and one strain of mouse (*M. musculus*).

Because this condition was met, EPA proceeded to Step 2.

Step 2: Calculation of a geometric mean requires at least three NOAEL results from the reproduction and growth effect groups.

Nine reproduction NOAEL results and 12 growth NOAEL results were reported from these studies.

Because this condition was met, EPA proceeded to Step 4.

Step 4: When the geometric mean of the NOAEL for reproduction and growth is higher than the lowest bounded LOAEL for reproduction, growth, or mortality, then the TRV is equal to the highest bounded NOAEL below the lowest bounded LOAEL.

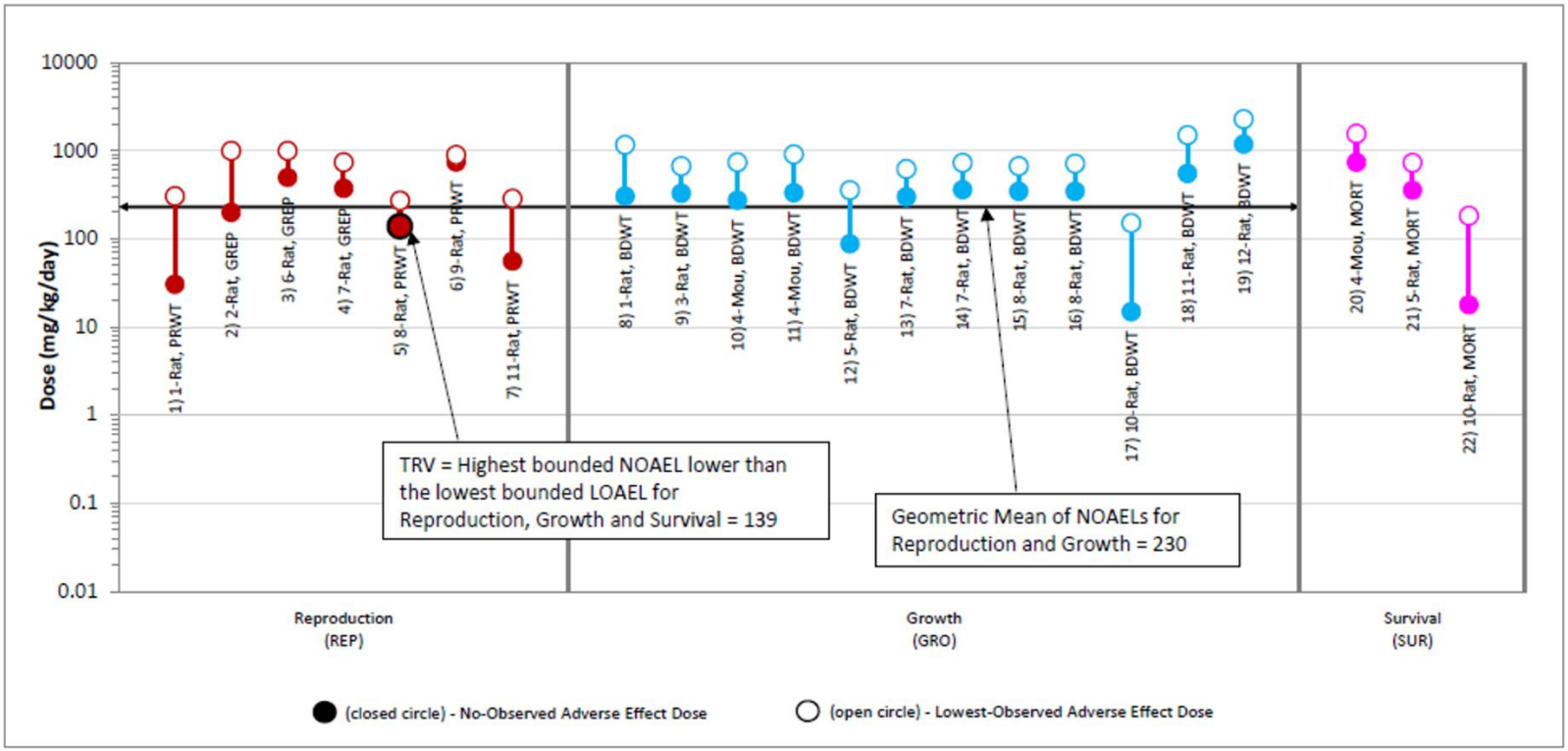
The geometric mean of NOAELs for reproduction and growth was 230 mg/kg-bw/day, which was higher than the lowest bounded LOAEL of 152 mg/kg-bw/day DINP from a study of reduced male body weight after 2 years of dietary exposure (Lington et al., 1997). The highest bounded NOAEL less than the lowest bounded LOAEL was 139 mg/kg-bw/day DINP (Waterman et al., 2000) a concentration corresponding to a reduction in second generation male rat body weight after 19 weeks of dietary exposure. Therefore, the terrestrial mammal TRV was **139 mg/kg-bw/day DINP in the diet**.

Summary of Environmental Hazard Thresholds

The effects of DINP on a generalized small mammal after consistent and prolonged ingestion of DINP in their diets (Table 6-1).

Table 6-1. Environmental Hazard Threshold for Aquatic and Terrestrial (TRV) Environmental Toxicity

Environmental Terrestrial Toxicity	Assessment Medium	Hazard Value or TRV
Mammal (TRV)	Dietary	139 mg DINP/kg-bw/day



Result number → 1) 10 - Rat, MORT

Reference number | Test Species | Effect Measure

Test Species Key: Mou - Mouse; Rat - Rat

Effect Measure Key:
BDWT - body weight changes
GREP - general reproduction
MORT - mortality
PRWT - progeny weight

Lowest-Observed Adverse Effect Dose
Paired values from same study when joined by line
No-Observed Adverse Effect Dose

Wildlife TRV Derivation Process

1) There are at least three results available for two test species within the growth, reproduction, and survival effect groups. There are enough data to derive a TRV.

2) There are at least three NOAEL results available in the growth and reproduction effect groups for calculation of a geometric mean.

3) The geometric mean of the NOAEL values for growth and reproductive effects equals 230 mg Di-isononyl phthalate/kg BW/day, which is greater than the lowest bounded LOAEL of 152 mg Di-isononyl phthalate/kg BW/day for reproduction, growth or survival.

4) The Mammalian wildlife TRV for Di-isononyl phthalate is equal to 139 mg Di-isononyl phthalate/kg BW/day, which is the highest bounded NOAEL below the lowest bounded LOAEL for reproduction, growth or survival.

Figure 6-1. Terrestrial Mammal TRV Derivation for DINP in Mammal Diets

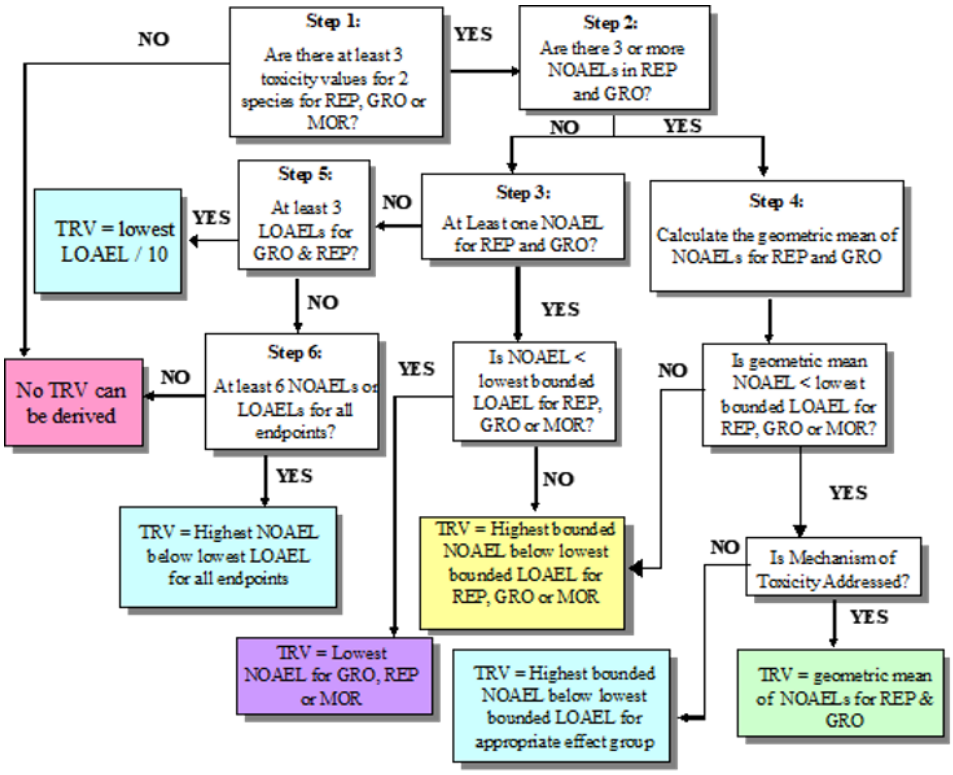


Figure 6-2. TRV Flow Chart

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Appendix A ENVIRONMENTAL HAZARD DETAILS

A.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 DINP, 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.

A.2 Weight of Scientific Evidence

After calculating the hazard thresholds that were carried forward to characterize risk, a narrative describing the weight of scientific evidence and uncertainties was completed to support EPA's decisions. The weight of scientific evidence fundamentally means that the evidence is weighed (*i.e.*, ranked) and weighted (*i.e.*, a piece or set of evidence or uncertainty may have more importance or influence in the result than another). Based on the weight of scientific evidence and uncertainties, a confidence statement was developed that qualitatively ranks (*i.e.*, robust, moderate, slight, or indeterminate) the confidence in the hazard threshold. The qualitative confidence levels are described below.

The evidence considerations and criteria detailed within (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 5-1 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 dataset. 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 rank correspond to the evidence table ranks of robust (+ + +), moderate (+ +), or slight (+), respectively. The evidence considerations are weighted based on professional judgment to obtain the overall confidence for each hazard threshold. In other words, the weights of each evidence property relative to the other properties are dependent on the specifics of the weight of scientific evidence and uncertainties that are described in the narrative and may or may not be equal. Therefore, the overall score is not necessarily a mean or defaulted to the lowest score. The confidence levels and uncertainty type examples are described below.

Confidence Levels

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

Types of Uncertainties

The following uncertainties may be relevant to one or more of the weights of scientific evidence considerations listed above and will be integrated into that property's rank in the evidence table (Table 5-1):

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

Table_Apx A-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 (2005) 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 analogue 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 dataset 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.

A.3 Strengths, Limitations, Assumptions, and Key Sources of Uncertainty for Environmental Hazard

Quality of the Database; Consistency; Strength (Effect Magnitude); and Precision

The database for the acute aquatic assessment consisted of 14 studies representing five fishes and four invertebrate species (Chen et al., 2014; Brown et al., 1998; Adams et al., 1995; EG & G Bionomics, 1984a, b; Springborn Bionomics, 1984a, b; EG & G Bionomics, 1983a, b). Twelve of the 14 studies received overall quality determinations of high, while the two other studies received overall quality determinations of medium increasing the overall strength of evidence for database quality (Chen et al., 2014; Springborn Bionomics, 1984a). Five fish species were represented with acute duration studies, and two aquatic invertebrate species were represented by three studies on *D. magna* and two studies on *M. bahia*, resulting in robust confidence in the overall quality of the database. All studies within the pool of reasonably available information resulted in similar findings of no acute adverse effects up to the limit of water solubility across all species and between vertebrate (Table 3-1) and invertebrate taxa (Table 3-2), resulting in robust confidence in the consistency of the database. Seven out of the eight acute aquatic studies were conducted with analytical verification of concentrations of DINP and details on precise results among treatment and control groups indicates robust confidence in the strength and precision of the exposure-response relationship.

The database for the acute benthic assessment consisted of two studies, both with overall quality determinations of high and representing *P. parthenogenetica* (Adams et al., 1995; EG & G Bionomics, 1984c). Moderate confidence in the overall quality of the database was determined, as the studies on benthic and epibenthic aquatic invertebrates produced two independent results. These studies demonstrated similar results within the same species tested (Table 3-2) leading to robust confidence assigned to the consistency consideration. Both studies were conducted with analytical verification of concentrations of DINP and provides precise detailed results of the data recorded, thereby providing robust confidence in the strength and precision of the exposure concentrations and associated response.

The database for the chronic aquatic assessment consisted of ten studies representing three fish species (Carnevali et al., 2019; Forner-Piquer et al., 2019; Forner-Piquer et al., 2018b; Forner-Piquer et al., 2018a; Santangeli et al., 2017; Patyna et al., 2006) and two aquatic invertebrates (Carnevali et al., 2019; Forner-Piquer et al., 2019; Forner-Piquer et al., 2018b; Forner-Piquer et al., 2018a; Santangeli et al., 2017; Patyna et al., 2006; Brown et al., 1998; Lake Superior Research Institute, 1997; Rhodes et al., 1995) and two aquatic invertebrates (Call et al., 2001; Brown et al., 1998; Lake Superior Research Institute, 1997; Rhodes et al., 1995). Four subchronic studies were conducted with 21-day aquatic exposures of DINP with two studies on zebrafish, two studies on *D. magna*, and two studies on the epibenthic amphipod, *H. azteca*. The remaining four studies were on dietary exposures of DINP to Japanese medaka (*O. latipes*) and gilthead sea bream (*S. aurata*). The dietary study conducted on *O. latipes* received an overall quality determination of high, while the remaining three dietary studies conducted on *S. aurata* received medium overall quality determinations. Studies conducted with 21-day aquatic exposures were of limited statistical power, observed inconsistent dose-response effects, were not analytically verified, and exceeded solubility (Forner-Piquer et al., 2018b; Santangeli et al., 2017). The 21-day feeding studies conducted on aquatic vertebrates displayed limited replication and sample sizes, relied on nominal concentration with no analytical verification of DINP within the feed, and did not demonstrate impacts on apical endpoints (Carnevali et al., 2019; Forner-Piquer et al., 2019; Forner-Piquer et al., 2018a). Moderate confidence was assigned to the overall quality of the chronic aquatic assessment database due to the low number of studies with apical endpoints from relative few species represented.

Both chronic duration studies conducted on aquatic invertebrates resulted in similar observations of no adverse effects from 21-day exposures of DINP, with one study observing presumed adverse effects from surface entrapment at the highest concentration tested (Rhodes et al., 1995) and the other study observing no adverse effects at an increased concentration of 1 mg/L DINP aided by the application of a dispersant Brown et al. (1998). Two studies with aquatic exposures of DINP to *D. rerio* for 21-days resulted in reproductive impacts (Forner-Piquer et al., 2018b; Santangeli et al., 2017), and two of the four studies conducted with dietary exposures of DINP were consistent in demonstrating adverse apical effects (Forner-Piquer et al., 2019; Patyna et al., 2006), indicating slight confidence regarding the consistency of effects on aquatic species from chronic exposure. Effect size, replication, and analytical verification of DINP within studies on chronic exposures to invertebrates and vertebrates was observed within studies such as Patyna et al. (2006) and Rhodes et al. (1995); however, low sample sizes and lack of analytical verification within other studies Santangeli et al. (2017) decreased evidence strength resulting in slight confidence in the strength and precision of the exposure-response relationship.

The database for the chronic benthic assessment consisted of one study representing sediment exposures of DINP to an amphibian species (*R. arvalis*) and two studies with an invertebrate species, *C. tentans* (Call et al., 2001; IVL, 2001; Lake Superior Research Institute, 1997). All three studies received overall quality determinations of high with studies on benthic invertebrates using subchronic 10-day exposures. Slight confidence was assigned to the overall quality of the database due to the limited number of studies, subchronic exposure duration, and the relevant species represented. No adverse effects were observed for the amphibian study, *R. arvalis*, throughout the 26-day exposures of DINP spiked sediment which was conducted from the embryo to tadpole stage (IVL, 2001). Moderate confidence was assigned to consistency for the chronic benthic assessment. Decreased confidence strength for the invertebrate chronic benthic assessment originates from the subchronic duration exposures to DINP spiked sediment within the two invertebrates studies, predominately following the OCSPP test guideline detailed within OCSPP 850.1735 Spiked Whole Sediment 10-Day Toxicity Test, Freshwater Invertebrates (Call et al., 2001; Lake Superior Research Institute, 1997). All three studies were conducted with analytical verification of concentrations of DINP, therefore moderate confidence was attributed to the strength and precision.

The database for the aquatic plant assessment consisted of three studies of algae, with two studies having overall quality determinations of high conducted on *S. capricornutum* (Adams et al., 1995; Springborn Bionomics, 1984c) and one study having an overall quality determination of medium conducted on the marine dinoflagellate, *K. brevis* (Liu et al., 2016). Slight confidence was assigned to the overall quality of the database due to the relatively limited number of studies and species represented. All studies were conducted with exposure durations of 96-hour and resulted in similar findings of no acute adverse effects on cell number up to the limit of water solubility across both species investigated (Table 3-3), providing robust confidence in the consistency in results of the algal assessment. Both studies conducted on *S. capricornutum* included analytical verification of DINP concentrations, while the study conducted on *K. brevis* reported nominal concentrations, indicating moderate confidence in the strength and precision consideration for the algal assessment.

The database for terrestrial mammals and the TRV derivation consisted of 12 studies that documented the DINP effects on laboratory rat and mouse reproduction, growth, and survival endpoints. EPA has moderate confidence in this database because the studies used model mammals to inform human health and not wildlife species. EPA has robust confidence in the consistency of the DINP effects on mammals because the effects were consistently observed at concentrations within the same order of magnitude. Similar strength and precision of the effects were observed across strains of rat and one mouse species,

resulting in a TRV that can be interpreted across many studies. Thus, EPA has robust confidence in these effects and the resultant TRV.

The database for terrestrial invertebrates consisted of one study (ExxonMobil, 2010) that found no mortality effects of soil DINP on *E. fetida*. EPA has slight confidence in quality of the database, consistency, and strength (effect magnitude) and precision because it is one study that represents one unbounded hazard soil concentration.

Biological Gradient/Dose-Response: Several acute toxicity tests for aquatic and benthic organisms were conducted with initial range finding tests followed by a definitive test with a single treatment concentration near the limit of solubility. In general, this approach would be interpreted to decrease the strength of the evidence for acute studies with aquatic and benthic organisms. However, given the fact that there is consistency among acute tests in the demonstration of no adverse effects up to the limit of solubility, EPA has moderate confidence in the biological gradient/dose-response for the acute toxicity assessments for aquatic and benthic organisms.

Among the six chronic studies conducted on fishes, two aquatic exposure studies included three or more treatment concentrations and had a medium overall quality determination (Forner-Piquer et al., 2018b) demonstrating evidence of concentration-response. A corresponding study from the same laboratory reported non-linear adverse effects at all five treatment concentrations for the number of eggs per female per day (Santangeli et al., 2017). The same study also reported adverse effects on gonadosomatic index at the two lowest and highest concentrations among three out of five aquatic DINP treatments. None of the chronic invertebrate studies with aquatic or benthic exposures reported any adverse effects resulting from DINP exposure. Rhodes et al. (1995) reported adverse effects at the highest concentration tested from 21-day DINP exposures to *D. magna*; however, as previously discussed impacts on mortality and subsequent reproduction were attributed to entrapment at the water surface. Moderate confidence in the biological gradient/dose-response consideration was assigned for the chronic toxicity assessments for aquatic organisms. Slight confidence in the biological gradient/dose-response consideration was assigned for the chronic assessments for benthic organisms due to a lack of DINP concentration gradients in these studies (Call et al., 2001; Lake Superior Research Institute, 1997).

Two of the three algal toxicity tests were conducted with initial range finding tests followed by a definitive test with a single treatment concentration near the limit of solubility, limiting the assessment of the biological gradient/dose-response consideration (Adams et al., 1995; Springborn Bionomics, 1984c). Liu et al. (2016) used five concentrations and a control for their investigations of acute DINP toxicity to the marine dinoflagellate, *K. brevis*, with no adverse effect on cell number at nominal concentrations compared to controls. Moderate confidence in the biological gradient/dose-response consideration was assigned for the algal assessment.

The database for terrestrial invertebrates consisted of one study (ExxonMobil, 2010) that found no mortality effects of soil DINP on *E. fetida*. EPA has slight confidence in Biological Gradient/Dose-response because only one test concentration was used. EPA has robust confidence in the dose-responses in rodent studies used to derive the TRV because they used gradients of DINP concentration in animal diets in their experimental designs.

Relevance (Biological; Physical/Chemical; Environmental): Acute aquatic studies similarly observed no adverse impacts of mortality or immobilization from acute DINP exposures within five species of fish and one invertebrate species. Test conditions for these species corresponded well with expected natural environmental conditions. Seven of the eight acute aquatic studies were conducted without the use of a

solvent and reported analytical verification of DINP treatment concentrations. Robust confidence in the relevance considerations was assigned for the acute aquatic assessment.

Acute benthic studies were represented by 48- and 96-hour exposure studies on the midge, *P. parthenogenetica*, (Adams et al., 1995; EG & G Bionomics, 1984c) The consistency in results among these independent studies on representative sediment-oriented species increases evidence strength for this consideration. All acute benthic studies were conducted without the use of a solvent and reported analytical verification of DINP treatment concentrations, providing moderate confidence in the relevance consideration for the acute benthic assessment.

Chronic aquatic studies are represented by studies with both invertebrates and vertebrates. Test concentrations were either not reported or not analytically verified for chronic aquatic studies with zebrafish (Forner-Piquer et al., 2018b; Santangeli et al., 2017) and chronic feeding studies with gilthead sea bream (Carnevali et al., 2019; Forner-Piquer et al., 2019; Forner-Piquer et al., 2018a). Because of this lack of analytical verification of concentrations, Moderate confidence in the relevance considerations was assigned for the chronic aquatic assessment.

Chronic benthic studies were limited to subchronic duration exposures conducted with the amphipod, *H. azteca*, the midge, *C. tentans*, and the moorfrog, *R. arvalis*, which are considered relevant study organisms for sediment toxicity testing. Although no adverse effects on mortality or development/growth were reported, these studies were conducted with 10-day exposures from DINP spiked sediment. Both studies conducted analytical verification of DINP within sediment, and one study (Lake Superior Research Institute, 1997) reported the corresponding concentration of DINP within porewater. Slight confidence in the relevance considerations was assigned for the chronic benthic assessment.

Algal toxicity studies are narrowly represented with the green algae, *S. capricornutum*, (Adams et al., 1995; Springborn Bionomics, 1984c) and the marine dinoflagellate, *K. brevis* (Liu et al., 2016). The two studies on *S. capricornutum* were conducted with analytical verification of DINP concentrations, while the remaining study on *K. brevis* did not perform analytical verification of the treatment concentrations but reported the purity, source, and nominal concentration of DINP. Based on the limited landscape of available studies for algal organisms and the duration of exposure, slight confidence in the relevance consideration was assigned for the algal assessment.

The database for terrestrial invertebrates consisted of one study (ExxonMobil, 2010) that found no mortality effects of soil DINP on earthworms. EPA has moderate confidence in its relevance (biological; physical/chemical; environmental) because soil concentrations were analytically verified, and earthworms are a relevant representative species. However, only one test concentration was used.

EPA has slight confidence in the relevance of the rodent studies and resultant TRV because they were conducted on non-wildlife species in highly controlled laboratory experiments, and they mainly found DINP effects after long term dietary exposures that may be unlikely in ecosystems. Additional uncertainties associated with laboratory to field variation in exposures to DINP are likely to have some effect on the hazard threshold; that is, formulated diets vs. natural forage diet for mammals (rats and mice).