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Environmental Hazard Assessment for Formaldehyde

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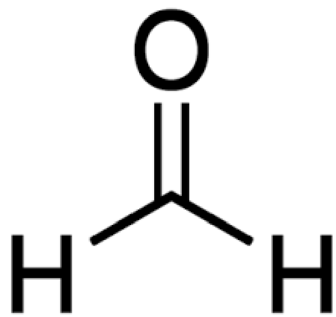

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EXECUTIVE SUMMARY

Ecological effects data were used to estimate the toxicity of formaldehyde and paraformaldehyde to surrogate species. All available ecotoxicity endpoints in EPA files are included herein for completeness regardless of whether a particular route/endpoint is or is not assessed for exposures. Endpoints were evaluated and extracted from (1) studies submitted by registrants under FIFRA to OPP, (2) federal laboratory data submitted and evaluated by OPP, and (3) those data from the public literature that underwent systematic review under TSCA by OPPT and were deemed high-ranking in data quality evaluation. Hazards from acute and chronic exposures are considered in this environmental hazard assessment.

Ecotoxicity data for formaldehyde currently available and evaluated to be acceptable for quantitative use include studies for freshwater fish (acute and chronic); freshwater invertebrates (acute); freshwater vascular plants; estuarine/marine fish (acute); estuarine/marine invertebrates (acute); terrestrial vertebrates (avian: acute and subacute; mammalian: oral and inhalation routes of exposure); and terrestrial vascular plants. Most aquatic toxicity data presented here were conducted using formalin (solution of water, 37% formaldehyde, and often 6–15% methanol). Given the volatility of formaldehyde, methanol is used to stabilize formaldehyde in aqueous solution and as such is expected to best represent aquatic exposure scenarios for formaldehyde. However, the use of this solution for toxicity tests results in some uncertainty in whether toxicity is directly related to formaldehyde alone, the combination of methanol and formaldehyde, or potential transformation products.

Formaldehyde is known to transform to methylene glycol, various oligomers, and paraformaldehyde (U.S. EPA, 2024a). Release of formaldehyde into the environment and intentional exposure to formaldehyde in toxicity studies will yield organismal exposure to all of these compounds due to the presence of water. As such, EPA considered the comparative toxicity of these compounds and determined that the formaldehyde toxicity data are protective or capture the toxicity of methylene glycol, oligomers, and paraformaldehyde.

Based on OPP ecotoxicity categories (see Table_Apx A-1), these data indicate that on an acute basis, formaldehyde is moderately toxic to birds, slightly to moderately toxic to freshwater fish, practically nontoxic to highly toxic to freshwater invertebrates depending on the species, moderately toxic to marine organisms, and moderately toxic to mammals via oral routes of exposure (see Table ES-1) (U.S. EPA, 2008). Chronic exposure toxicity was an order of magnitude lower (*i.e.*, more toxic) than acute exposure toxicity values for freshwater fish. Additionally, given the lack of chronic exposure toxicity data for the most acutely sensitive aquatic invertebrate (*i.e.*, ostracod) to formaldehyde, EPA used an acute-to-chronic ratio to estimate chronic exposure toxicity to this freshwater invertebrate. Results suggest that chronic sublethal aquatic invertebrate toxicity to formaldehyde is also approximately an order of magnitude below acute exposure toxicity values. Reliable high-quality data were not available for terrestrial invertebrates or nonvascular plants. All ecotoxicity endpoints tabulated below are adjusted to represent toxicity to formaldehyde alone.

Table ES-1. Ecological Effects Endpoints Selected for Formaldehyde

Receptor Group	Exposure Scenario	Toxicity Endpoint(s)	Toxicity Category	Citation or MRID
Freshwater fish	Acute	LC50 = 9.35 mg/L[a]	Moderately toxic	(Fajer-Avila et al., 2003)
	Chronic	NOAEC = 0.62 mg/L[a] (21% reduction in weight gain, $p > 0.05$) LOAEC = 1.25 mg/L[a] (40% reduction in weight gain)	N/A	(Omoregie et al., 1998)
Freshwater invertebrates	Acute	LC50 = 0.32 mg/L[a]	Highly toxic	(Bills et al., 1977), MRID 00132485
	Chronic	0.063 mg/L[a]	N/A	ACR[b] from (Bills et al., 1977), MRID 00132485
Freshwater vascular plants	N/A	EC50 = 0.18 mg/L[a] (biomass) LOAEC = 0.1 mg/L[a] (25% reduction in biomass) NOAEC < 0.1 mg/L[a]	N/A	(Singh et al., 2008)
Freshwater non-vascular plants	N/A	No data	N/A	N/A
Estuarine/marine fish	Acute	LC50 = 2.92 mg/L[a]	Moderately toxic	(Takayanagi et al., 2000)
	Chronic	No data	N/A	N/A
Estuarine/marine invertebrates	Acute	LC50 = 1.96 mg/L[a]	Moderately toxic	(Fajer-Avila et al., 2003)
	Chronic	No data	N/A	N/A
Birds	Acute	LD50 = 292.3 mg/kg-bw	Moderately toxic	MRID 00148774
	Subacute dietary	LC50 > 1,850 mg/kg-diet	Slightly toxic	MRID 00148775
Mammals	Acute oral	LOAEC = 3.1 mg/kg/day NOAEC < 3.1 (based on pup weight)	Moderately toxic	MRID 00143291
	26-week inhalation	LOAEC = 3.0 ppm (3.68 mg/m^3) NOAEC = 1.0 ppm (1.23 mg/m^3)	N/A	MRID 00149755
Terrestrial plants	N/A	438 $\mu g/m^3$ (based on growth)	N/A	(Mutters et al., 1993)
bw = body weight; LOAEC = lowest-observed-adverse-effect concentration; MRID = Master Record Identifier MRID; NOAEC = no-observed-adverse-effect concentration [a] mg/L = mg per liter formaldehyde adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity unless otherwise noted. [b] ACR = acute-to-chronic ratio; used to estimate chronic toxicity from acute toxicity data, or vice versa.				

1 INTRODUCTION

This environmental hazard assessment of formaldehyde is a joint assessment that will serve as a reference for both OPP and OPPT as part of their ongoing risk assessment and regulatory efforts. The properties listed in this assessment may differ from those previously published by OPP and OPPT.

1.1 Risk Evaluation Scope

The TSCA risk evaluation of formaldehyde comprises several human health and environmental modules and two risk assessment documents—the ecological risk assessment and the human health risk assessment. A basic diagram showing the layout of these modular assessments and their relationships is provided in Figure 1-1. This environmental hazard assessment is shaded blue. In some cases, modular assessments were completed jointly under TSCA and FIFRA. This is one such module; the others are shown in dark gray.

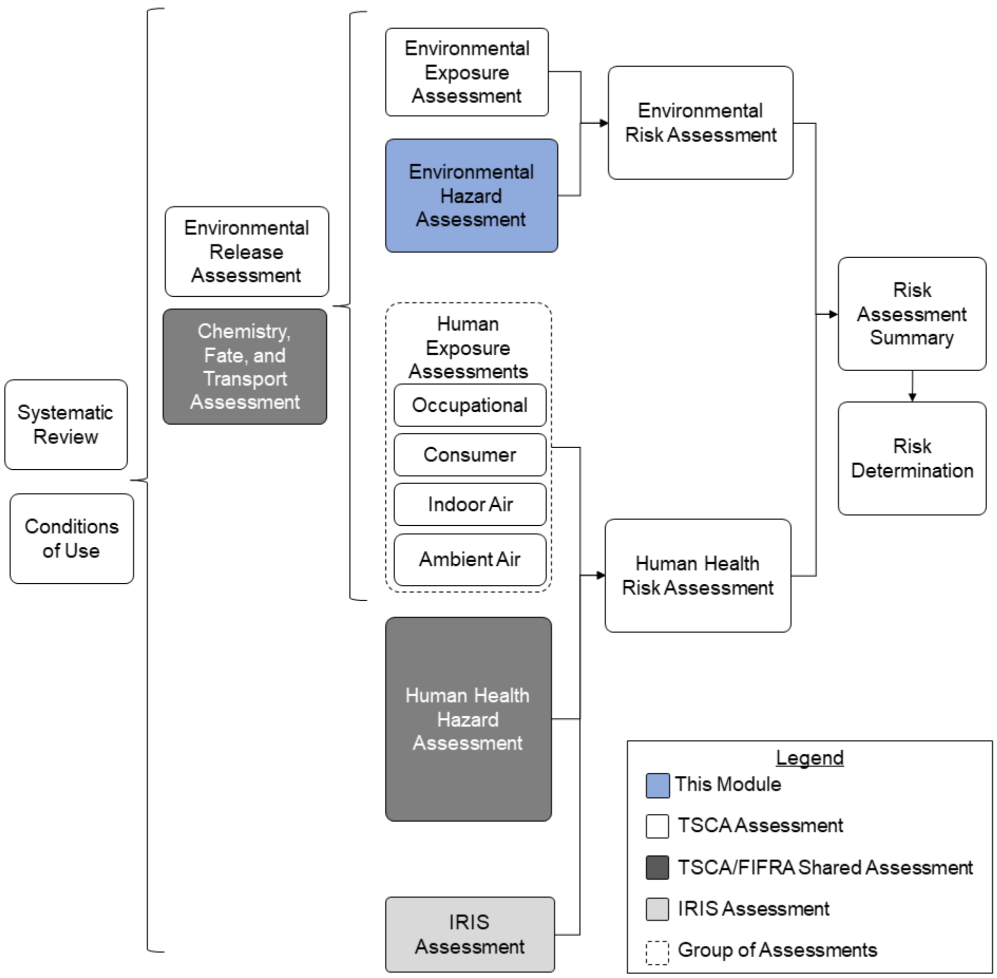


Figure 1-1. Risk Evaluation Document Summary Map

This environmental hazard module is a TSCA/FIFRA shared assessment.

1.2 Changes between Draft and Revised Assessment

No major updates were made to this technical support document between the draft and revised assessment.

1.3 Approach and Methodology

EPA reviewed potential environmental health hazards associated with formaldehyde. In addition, the relevant isomer paraformaldehyde was also reviewed. EPA utilized two major sources of environmental hazard data (listed below) to characterize the environmental hazards of formaldehyde and paraformaldehyde to surrogate species representing various receptor groups, including freshwater fish (acute and chronic); freshwater invertebrates (acute); freshwater vascular plants; estuarine/marine fish (acute); estuarine/marine invertebrates (acute); and terrestrial vertebrates (avian: acute and subacute; mammalian: oral routes of exposure). Reliable high-quality data were not available for terrestrial invertebrates or aquatic nonvascular plants and were limited across terrestrial receptor groups.

- ***High-Quality Studies from OPPT Systematic Review:*** TSCA requires that EPA use data and/or information in a manner consistent with the best available science and that the Agency base decisions on the weight of scientific evidence. To meet the TSCA science standards, OPPT applies a systematic review process to identify data and information across taxonomic groups for both aquatic and terrestrial organisms with a focus on apical endpoints (*e.g.*, those affecting survival, growth, or reproduction). The data collection, data evaluation, and data integration stages of the systematic review process are used to develop the hazard assessment to support the integrative risk characterization. EPA completed the review of environmental hazard data/information sources during risk evaluation using the data quality review evaluation metrics and the rating criteria described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances, Version 1.0: A Generic TSCA Systematic Review Protocol with Chemical-Specific Methodologies* (also called the 2021 Draft Systematic Review Protocol Supporting TSCA Risk Evaluations). *(*U.S. EPA, 2021*)*. Studies identified and evaluated by OPPT were assigned an overall quality level of high, medium, low, or uninformative. Because data on toxicity of formaldehyde are numerous and in some instances vary substantially, EPA systematically evaluated all data for this hazard characterization but relies upon only high-quality studies for purposes of risk characterization (U.S. EPA, 2024b).
- ***Acceptable and Supplemental Studies Identified through the OPP Review Process:*** Under FIFRA, EPA can require data to support an application for registration of a pesticide under section 3(c)(1)(F) or to support the continued registration of a pesticide under section 3(c)(2)(B). Under section 6(a)(2), pesticide registrants are required to inform EPA of any relevant information related to their products, including new studies or incidents of adverse effects. OPP data requirements for antimicrobial pesticides are identified in 40 CFR Part 158W, but EPA has the authority to require additional data as necessary. Studies submitted in response to FIFRA requirements are conducted under and evaluated with a series of internationally harmonized and scientifically peer-reviewed study protocols. These protocols are designed to maintain a high standard of scientific quality and ensure that study results can be repeated. They also ensure consistent review of studies. In addition to studies submitted by the registrant, OPP may also rely on studies identified in the open literature or conducted by other federal agencies if they are of sufficient scientific quality. The *Evaluation Guidelines for Ecological Toxicity Data in the Open Literature* (U.S. EPA, 2011) outlines how open literature is searched and reviewed in OPP to evaluate the quality and utility of open literature studies in a transparent and systematic way. Studies reviewed according to the OPP process are identified with Master Record Identifier (MRID) numbers throughout this document.

When empirical data were not readily available for formaldehyde, an ecological structure activity relationship (ECOSAR) analysis was used to estimate toxicity to qualitatively characterize ecotoxicity hazards. If empirical data were available, data were relied upon unless otherwise noted. Details of ECOSAR analyses are described in Section A.3 and Appendix A.

2 ENVIRONMENTAL HAZARD

2.1 Comparative Toxicology

In water, formaldehyde is readily hydrated to methylene glycol, which exists in equilibrium with various oligomers and paraformaldehyde. Therefore, these compounds can occur simultaneously with any introduction of formaldehyde to water, though the methylene glycol form is typically dominant (U.S. EPA, 2024). The presence of one or more of these chemicals in water led EPA to evaluate the relative toxicity of methylene glycol and paraformaldehyde to formaldehyde.

Although an abundance of data on formaldehyde toxicity is available, there are limited toxicity data on paraformaldehyde. However, the available information on paraformaldehyde still allows EPA to conduct a comparative assessment of toxicity between formaldehyde and paraformaldehyde. For instance, formaldehyde toxicity to aquatic invertebrates ranged from LC50s (lethal concentrations to half of the tested population) of 0.32 mg/L (Bills et al., 1977) (MRID 00132485) to 251.79 mg/L (Bills et al., 1977) (MRID 00132485). Two paraformaldehyde toxicity studies had LC50s that fell within this range. These studies included a 48-hour acute assay with oyster embryos (C*rassostrea virginica*) with LC50s between 2.9 and 5.1 mg/L of paraformaldehyde (Cook, 1975) (MRID 00126395) and a 96-hour aquatic invertebrate (*Penaeus duorarum*) study with an LC50 of 28.2 mg/L of paraformaldehyde (Cook, 1975) (MRID 00126395). Acute fish toxicity (96-hour) data for formaldehyde and paraformaldehyde were available for the same species (rainbow trout [*Oncorhynchus mykiss*] and bluegill sunfish [*Lepomis macrochirus*]). A comparison of these data showed similar toxicity for paraformaldehyde (rainbow trout: LC50 = 49.02, 95% C.I. 41.74–55.66 mg paraformaldehyde/L; bluegill sunfish: LC50 = 38.55, 95% C.I. 32–49 mg paraformaldehyde/L) and formaldehyde (rainbow trout: LC50s = 35.58 to 70.56 mg/L; bluegill sunfish: LC50 = 30.16 mg/L) (Edmundson, 1975) (MRID 00101857, MRID 00101865) (Bills et al., 1977) (MRID 00132485) (see Table 2-1).

Although acute fish ecotoxicity endpoints for paraformaldehyde were not used quantitatively, they support the conclusion that formaldehyde and paraformaldehyde have similar toxicity (Table 2-1). In aquaculture, formaldehyde solutions containing paraformaldehyde due to spontaneous formation also have similar fish toxicity to formaldehyde not containing paraformaldehyde (Howe et al., 1995), further supporting the Agency assumption that formaldehyde and paraformaldehyde are equitoxic at environmentally relevant concentrations.

Table 2-1. Comparison of Formaldehyde and Paraformaldehyde Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in 96-Hour Acute Exposure Toxicity Assays (LC50, Mortality)

Compound	Chemical Purity (%) [a]	Test Species	Hazard Value(s) (mg/L) [b]	Citation, MRID [c]
Formaldehyde	37	Rainbow trout	35.6 36.5 70.6	(Bills et al., 1977), MRID 00132485, (Howe et al., 1995)
Formaldehyde		Bluegill sunfish	30.2	(Bills et al., 1977), MRID 00132485
Paraformaldehyde	91	Rainbow trout	49.0	(Edmundson, 1975), MRID 00101857, MRID 00101865
Paraformaldehyde		Bluegill sunfish	38.6	(Edmundson, 1975), MRID 00101857, MRID 00101865

[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations
[b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] OPP classified MRID 00132485 as acceptable for quantitative use and MRIDs 00101857 and 00101865 as supplemental qualitative

As there were no data available on the toxicity of methylene glycol to aquatic or terrestrial organisms, EPA conducted an ECOSAR analysis to determine if formaldehyde and methylene glycol have ECOSAR chemical classes in common that may help predict the toxicity of methylene glycol. Formaldehyde ECOSAR toxicity predictions using class results for aldehydes (mono) were generally in agreement with measured formaldehyde toxicity reported throughout this hazard characterization (Table 2-2), often within an order of magnitude or falling within the range of measured values. However, ECOSAR predictions for methylene glycol, using SMILES code C(O)O, did not provide results for the same chemical class (aldehydes) as formaldehyde, and only included class results for Neutral Organics. ECOSAR predictions for formaldehyde toxicity under chemical class Neutral Organics were two or more orders-of-magnitude less sensitive than measured formaldehyde toxicity; therefore, predicted toxicity results for methylene glycol under this class were not considered reliable (Table 2-2). In the absence of reliable data or predictions for methylene glycol toxicity and given the similar chemical properties between formaldehyde and methylene glycol, the Agency assumed formaldehyde toxicity is protective or captures the methylene glycol toxicity in environmental media.

Table 2-2. Comparison of Measured Hazard Values to Predicted ECOSAR Hazard Values of Formaldehyde and Methylene Glycol[a]

Organism	Endpoint	Measured Formaldehyde Hazard Value[b] (mg /L)	Predicted Formaldehyde Hazard Value (mg/L) Aldehydes (Mono)	Predicted Formaldehyde Hazard Value (mg/L) Neutral Organics	Predicted Methylene Glycol Hazard Value (mg /L) Neutral Organics
FW Fish	LC50	9.35	11.2	748	12,700
	Chronic	0.62	3.62	61.1	913
SW Fish	LC50	2.92	–	933	15,700
	Chronic	–	–	37	310
FW Invertebrate (Daphnid)	LC50	5.29	12.0	365	5,560
	Chronic	1.04	0.098	23.3	265
SW Invertebrate	LC50	1.96 (based on pearl oyster)	–	2,120 (based on mysid)	77,200 (based on mysid)
	Chronic	–	–	299	15,300
Green Algae	EC50	–	5.87	145	1,430
	Chronic	–	1.78	27	210
Earthworm	LC50	–	–	77.4	163

EC = effect concentration; FW = freshwater; SW = saltwater
[a] See also Section A.3
[b] Selected measured formaldehyde hazard values are the most sensitive endpoint for all except freshwater invertebrates, where *Daphnia* values were used for comparison to *Daphnia*-specific predictions.
ECOSAR-predicted chronic values represent the geometric mean of the no-observable-adverse-effect concentration (NOAEC) and lowest-observable-adverse-effect concentration (LOAEC); measured chronic values are NOAECs.

Due to the equilibrium reactions, the presence of methylene glycol, various oligomers, and paraformaldehyde are all likely at a formaldehyde release point. The comparable toxicity among formaldehyde, methylene glycol, and paraformaldehyde at environmentally relevant concentrations support the use of formaldehyde as an assumed proxy for the toxicity of methylene glycol and paraformaldehyde in aquatic media for aquatic taxa. Thus, formaldehyde is the focus of this environmental hazard characterization.

2.2 Aquatic Species Hazard

To characterize formaldehyde hazards to aquatic species, EPA examined ecotoxicity studies for 13 freshwater fish species, 6 freshwater invertebrate species, 1 aquatic vascular plant species, 1 estuarine/marine fish species, and 1 estuarine/marine invertebrate species. These studies were classified by OPPT as high quality and/or classified as acceptable for quantitative use by OPP and are included in this environmental hazard characterization.

Results of these studies indicate that on an acute basis, formaldehyde is slightly to moderately toxic to freshwater fish, practically nontoxic to highly toxic to freshwater invertebrates, and moderately toxic to marine organisms (U.S. EPA, 2008). On a chronic basis, the highest tested concentrations that resulted in no adverse effects to freshwater fish was 0.62 mg/L. EPA also used an acute-to-chronic ratio to estimate chronic exposure toxicity to the most acutely sensitive freshwater invertebrate (*i.e.*, ostracod), and ECOSAR predictions for formaldehyde toxicity under the class Aldehyde (Mono) to qualitatively characterize missing ecotoxicity endpoints for other aquatic organisms (Table 2-2). The most sensitive ecotoxicity endpoints for each receptor group are bolded in tables below. However, it should be noted that nearly all studies reported herein were conducted with formalin—a solution of 37 percent formaldehyde, water, and 6 to 15 percent methanol. Although this is a common solution for the distribution of formaldehyde, the use of it in toxicity studies causes some uncertainty in whether toxicity is directly related to formaldehyde alone or the combination of methanol and formaldehyde and its transformation products. All ecotoxicity endpoints reported are based on concentrations of formaldehyde alone unless otherwise noted. Lastly, acceptable data to characterize formaldehyde hazards to aquatic nonvascular plants were not available.

2.2.1 Freshwater Fish

Acute fish toxicity to formaldehyde ranged from 9.35 mg/L in the most sensitive species, Atlantic sturgeon (*Acipenser oxyrhynchus*) (King and Farrell, 2002) to 163.7 mg/L in the least sensitive species, mosquitofish (*Gambusia affinis*) in 96-hour LC50 assays (Table 2-3). Exposure time in acute studies generally increased toxicity over 6-, 24-, and 96-hour exposure durations in channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) (Howe et al., 1995). Across OPPT high-quality and OPP quantitative studies, 12 species were represented in the evaluation of acute exposure toxicity to fish. The Agency conducted an SSD analysis to determine the calculated hazardous concentration for 5 percent of species (HC05) (see Section A.3 and Appendix A). This information was also used to provide insight on predicted formaldehyde toxicity to higher percentages of the freshwater fish receptor group (*e.g.*, hazard concentration (HC50); Section A.3 and Appendix A). Freshwater fish were the only receptor group with sufficient data to conduct this analysis.

Several models were fit to acute exposure toxicity LC50 values and compared using Akaike's Information Criteria (AIC_c) corrected for small sample size (Burnham and Anderson, 2002). The logistic model had the lowest AIC_c value, and therefore best fit the data. The HC05 predicted from the logistic model for the freshwater fish receptor group was 11.47 mg/L ($p = 0.8152$; 7.96–14.97 mg/L 95% CI) (Table_Apx A-2, Table_Apx A-3). This result was nearly identical, though slightly less sensitive, than the most sensitive ecotoxicity endpoint from this receptor group (9.35 mg/L). Based on ecotoxicity categories (Table_Apx A-1), formaldehyde ranges from practically nontoxic to moderately toxic across freshwater fish species with the most sensitive species tested being moderately toxic.

Table 2-3. Acute Freshwater Fish Toxicity of Formaldehyde

Test Species	Duration (hours)	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Toxicity Category	Citation, MRID [c]
Channel catfish	24	LC50	37	28.19	Slightly toxic	(Howe et al., 1995)
Rainbow trout	24	LC50	37	70.56	Slightly toxic	(Howe et al., 1995)
Atlantic sturgeon	**96**	**LC50**	**37**	**9.35**	**Moderately toxic**	**(King and Farrell, 2002)**
Channel catfish	96	LC50	37	10.55	Slightly toxic	(Howe et al., 1995)
Black bullhead	96	LC50	37	18.73	Slightly toxic	(Bills et al., 1977), MRID 00132485
Channel catfish	96	LC50	37	19.84	Slightly toxic	(Bills et al., 1977), MRID 00132485
Fathead minnow	96	LC50	90	24.50	Slightly toxic	(Brooke, 1987)

Test Species	Duration (hours)	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Toxicity Category	Citation, MRID [c]
Bluegill sunfish	96	LC50	37	30.16	Slightly toxic	(Bills et al., 1977), MRID 00132485
Lake trout	96	LC50	37	30.16	Slightly toxic	(Bills et al., 1977), MRID 00132485
Rainbow trout	96	LC50	37	35.58	Slightly toxic	(Bills et al., 1977), MRID 00132485
Rainbow trout	96	LC50	37	36.49	Slightly toxic	(Howe et al., 1995)
Smallmouth bass	96	LC50	37	41.01	Slightly toxic	(Bills et al., 1977), MRID 00132485
Largemouth bass	96	LC50	37	43.12	Slightly toxic	(Bills et al., 1977), MRID 00132485
Atlantic salmon	96	LC50	37	52.17	Slightly toxic	(Bills et al., 1977), MRID 00132485
Green sunfish	96	LC50	37	52.17	Slightly toxic	(Bills et al., 1977), MRID 00132485
Mosquitofish	96	LC50	100	163.70	Practically nontoxic	(McCorkle, 1979)

[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations
[b] mg/L = mg formaldehyde per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] All listed studies evaluated mortality and are high-ranking studies from OPPT systematic review or OPP acceptable studies. All OPP studies are identified with MRID.
The most sensitive endpoint identified is bolded.

Only one high-quality study evaluated chronic exposure toxicity to freshwater fish (Omoregie et al., 1998). A 12-week exposure of Nile tilapia (*Oreochromis niloticus*) fingerlings had reduced fish weight gain with a NOAEC of 0.62 mg/L and the LOAEC of 1.25 mg/L (Table 2-4). These data suggest that chronic exposure toxicity of formaldehyde to freshwater fish is approximately an order of magnitude lower than acute exposure toxicity.

Table 2-4. Chronic Freshwater Fish Toxicity of Formaldehyde

Test Species	Duration	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Effect	Citation[c]
Nile tilapia	12 weeks	LOAEC	40	1.25	40% reduction in weight gain	(Omoregie et al., 1998)
Nile tilapia	**12 weeks**	**NOAEC**	**40**	**0.62**	**21% reduction in weight gain ($p > 0.05$)**	**(Omoregie et al., 1998)**

[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations
[b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] High-ranking study from OPPT systematic review
The most sensitive endpoint identified is bolded.

2.2.2 Freshwater Invertebrates

Acute freshwater invertebrate toxicity to formaldehyde varied over several orders of magnitude depending on species (Table 2-5). The most sensitive organism, ostracods (*Cypridopsis* sp.), had 50 percent mortality at 0.32 mg/L (96-hour). In contrast, common backswimmers (*Notonecta* sp.) had 50 percent mortality at 251.8 mg/L (96-hour) (Bills et al., 1977) (MRID 00132485). Based on OPP toxicity categories, formaldehyde was practically nontoxic to highly toxic to freshwater invertebrates on an acute basis (U.S. EPA 2022).

Table 2-5. Acute Freshwater Invertebrate Toxicity of Formaldehyde (LC50, Immobility)

Test Species	Duration (hours)	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Toxicity Category	Citation, MRID[c]
Ostracod	**96**	**LC50**	**37**	**0.32**	**Highly toxic**	**(Bills et al., 1977), MRID 00132485**
Daphnid	48	LC50	37	5.29	Moderately toxic	(Natella, 1975), MRID 00148772
Gastropod	96	LC50	37	28.04	Slightly toxic	(Bills et al., 1977), MRID 00132485
Clam	96	LC50	37	38.00	Slightly toxic	(Bills et al., 1977), MRID 00132485
Grass shrimp	96	LC50	37	140.2	Practically nontoxic	(Bills et al., 1977), MRID 00132485
Backswimmer	96	LC50	37	251.8	Practically nontoxic	(Bills et al., 1977), MRID 00132485

[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations
[b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] High-ranking studies from OPPT systematic review or OPP acceptable studies. All OPP studies are identified with MRID
The most sensitive endpoint identified is bolded.

Mortality of freshwater invertebrates with longer-term exposure durations (*e.g.,* 21 days) to formaldehyde in water (Table 2-6) was measured in sludge worms (*Tubifex tubifex*) and water fleas (*Daphnia magna*). Sludge worm toxicity was 0.39 mg/L LC50 with 21 days of exposure, though shorter durations of 7 days and 14 days measured LC50 at 0.73 and 0.48 mg/L, respectively (Singh et al., 2008). Growth was also measured, showing an increase in the control through the study from 102 to 155 percent from 7 days to 21 days, respectively. Conversely, formaldehyde treated sludge worms lost weight in response to exposure duration and dose. At the 21-day exposure duration, the control group had a 155 percent increase in growth, while the lowest tested formaldehyde concentration (0.1 mg/L) showed an approximate 10 percent decline in growth. These results demonstrate a NOAEC for sludge worms less than 0.1 mg/L and a LOEAC of 0.1 mg/L. Because the NOAEC from this study was not determinative, this value cannot be used quantitatively but can be used qualitatively for risk characterization.

A 21-day formaldehyde exposure study on *Daphnia magna* reproduction and mortality reported an EC50 of 9.6 mg/L (95% CI 7.5–12.8 mg/L). The most sensitive reported sublethal adverse effect from this study was age at first reproduction with a NOAEC of 1.04 mg/L and a LOAEC of 2.56 mg/L. While this study does provide a chronic NOAEC, the endpoint is greater than the most sensitive acute endpoint for this receptor group. However, this endpoint could be used to calculate an ACR for *Daphnia magna* (5.29/1.04 = 5.08), that was then used to estimate the chronic NOAEC for the most sensitive acute freshwater invertebrate (ostracod LC50 = 0.32). The results, based on this ACR, was a NOAEC for ostracod of 0.063 mg/L (0.32/5.08 = 0.063).

Table 2-6. Chronic Freshwater Invertebrate Toxicity of Formaldehyde

Test Species	Duration (days)	Endpoint(s)	Chemical Purity (%) [a]	Hazard Value(s) (mg/L) [b]	Effect	Citation [c]
Sludge worm	7	LC50	100	0.73	Mortality	(Singh et al., 2008)
Sludge worm	14	LC50	100	0.48	Mortality	(Singh et al., 2008)
Sludge worm	21	LC50	100	0.39	Mortality	(Singh et al., 2008)
Sludge worm	21	NOAEC LOAEC	100	<0.1 0.1	Growth	(Singh et al., 2008)
Water flea	21	EC50	40	9.6	Reproduction	(Institut, 2008)
Water flea	21	NOAEC LOAEC	40	1.04 2.56	Age at first reproduction	(Institut, 2008)
Ostracod	**21**	**NOAEC**	**N/A**	**0.063**	**N/A**	**ACR**[d]

[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations
[b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] High-ranking studies from OPPT systematic review
[d] An ACR was used to estimate the chronic endpoint for the most sensitive freshwater invertebrate, ostracods (*Cypridopsis* sp.). An ACR of 5.29 is derived from the acute and chronic studies of (Natella, 1975), MRID 00148772 and (Institut, 2008) for *Daphnia magna*. ACR = 5.29/1.04 = 5.08. The NOAEC for ostracod was estimated using the following equation: NOAEC = acute ostracod/ACR = 0.32/5.08 = 0.063.
The most sensitive endpoint identified is bolded.

2.2.3 Freshwater Plants

There was only one high-quality study evaluating toxicity of formaldehyde to freshwater vascular plants (Singh et al., 2008) (Table 2-7). Duckweed (*Lemnoideae*) growth was inhibited at 0.18 mg /L (IC50) beginning at 9 days following a single exposure with continued inhibition through 21 days of exposure in artificial mesocosms. A significant ($p < 0.05$) 25 percent reduction in biomass was also measured at the lowest tested dose in this study, resulting in a LOAEC of 0.1 mg/L (NOAEC < 0.1 mg/L). Acceptable data to characterize formaldehyde hazards to nonvascular plants were not available. Two open literature studies on nonvascular plants (MRIDs 50825102 and 50825103) were submitted to and evaluated by OPP. EPA classified these studies as supplemental qualitative largely because the test concentrations and purity of the test substance were not reported. This classification allows only for the qualitative use of these data in the evaluation of formaldehyde hazards to nonvascular plants. The most sensitive apical endpoint for formaldehyde reported from these studies was for algal species *Desmodesmus subspicatus* with an inhibitory concentration (IC50) of 3.48 mg/L, although the purity of the test substance was not reported. Predicted estimates of formaldehyde toxicity to green algae using ECOSAR provided data on nonvascular plant toxicity, with results indicating IC50 toxicity at 5.87 mg/L to 1.78 mg/L.

Table 2-7. Freshwater Plant Formaldehyde Toxicity

Test Species	Duration	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Effect	Citation [c]
Duckweed	**21 days**	**IC50**	**100**	**0.18**	**Biomass**	**(Singh et al., 2008)**
		LOAEC	**100**	**0.1**	**Biomass (25% reduction)**	
		NOAEC	**100**	**<0.1**	**N/A**	

[a] % = % formaldehyde in the test substance (*e.g.*, formalin) used to prepare test concentrations
[b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] High-ranking study from OPPT systematic review.
The most sensitive endpoint identified is bolded.

2.2.4 Marine/Estuarine Fish

Across taxa, few high-quality studies were found evaluating formaldehyde toxicity to marine/estuarine fish (Table 2-8). One high-quality study evaluated acute vertebrate toxicity to marine fish (bullseye puffer, *Sphoeroides annulatus*) with LC50 values ranging from 2.92 mg/L (72-hour mortality) to 3.22 mg/L (48-hour mortality) (Fajer-Avila et al., 2003). These data suggest that marine fish may be more sensitive to formaldehyde than freshwater fish, though more data are needed for evaluation. OPP toxicity categories classify formaldehyde as moderately toxic to estuarine/marine fish.

Table 2-8. Acute Exposure Toxicity of Formaldehyde on Marine/Estuarine Fish

Test Species	Duration (hours)	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Toxicity Category	Citation [c]
Bullseye puffer fish	**72**	**LC50**	**37**	**2.92**	**Moderately toxic**	**(Fajer-Avila et al., 2003)**
Bullseye puffer fish	48	LC50	37	3.22	Moderately toxic	(Fajer-Avila et al., 2003)

[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations
[b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity
[c] High-ranking study from OPPT systematic review.
The most sensitive endpoint identified is bolded.

2.2.5 Marine/Estuarine Invertebrates

Across taxa, there were also limited high-quality studies that evaluated formaldehyde toxicity to marine/estuarine invertebrates (Table 2-9). One high-quality study evaluated acute invertebrate toxicity to marine pearl oyster, *Pinctada fucata*. Exposure to formaldehyde in water yielded 50 percent mortality to pearl oysters at concentrations ranging from 1.96 to 2.85 mg/L depending on water temperature (25 and 20 °C, respectively; 96-hour mortality)(Takayanagi et al., 2000). OPP toxicity categories classify formaldehyde as moderately toxic to estuarine/marine invertebrates.

Table 2-9. Acute Exposure Toxicity of Formaldehyde on Marine/Estuarine Invertebrates

Test Species	Duration (hours)	Endpoint	Chemical Purity (%) [a]	Hazard Value (mg/L) [b]	Toxicity Category	Citation[c]
Pearl oyster	**96**	**LC50**	**37**	**1.96**	**Moderately toxic**	**(Takayanagi et al., 2000)**
[a] % = % formaldehyde in the test substance (*e.g.*, formalin, 37%) used to prepare test concentrations [b] mg/L = mg per liter as active ingredient adjusted for chemical purity by multiplying the measured hazard value by the percent chemical purity [c] High-ranking study from OPPT systematic review. The most sensitive endpoint identified is bolded.						

2.3 Terrestrial Species Hazard

To characterize formaldehyde hazards to terrestrial species, EPA examined dietary toxicity studies for three avian species, oral toxicity to three mammalian species, and air exposure toxicity to five plant species. These studies were classified by OPPT as high quality and/or classified as acceptable for quantitative use by OPP and are included in this hazard characterization.

Formaldehyde is known to transform to methylene glycol, various oligomers, and paraformaldehyde in the aquatic environment (U.S. EPA, 2024a). Oral exposure to all of these formaldehyde transformation products is expected for terrestrial taxa given the most likely route of oral exposure from formaldehyde releases is through drinking water. Limited toxicity data were available for formaldehyde transformation products in terrestrial taxa, with only one study available for mammals on paraformaldehyde. Paraformaldehyde ecotoxicity data for terrestrial mammals had a more sensitive LOAEC than formaldehyde when comparing a 28-day (Holtzman rats; LOAEC = 279 mg/kg/day; MRID 00124677) and a 90-day (Sprague-Dawley rats; LOAEC = 95 mg/kg/day; (Johannsen et al., 1986)) toxicity test for rats. However, it is unclear whether the difference in the results for these studies are from the different species of rat tested, the different durations of exposure, or the different test substances. Additionally, no acceptable OPP data or high-ranking OPPT studies were available to evaluate formaldehyde or paraformaldehyde toxicity to terrestrial invertebrates including pollinators. Therefore, there is uncertainty in the relative toxicity of formaldehyde and paraformaldehyde to terrestrial taxa.

Data from these studies suggest that formaldehyde is moderately toxic to birds and mammals on an acute basis through diet and slightly toxic to birds on a subacute dietary basis. Terrestrial plants exposed to formaldehyde through air had a NOAEC of 438 $\mu g/m^3$. Ecotoxicity data are described below with the most sensitive endpoints for each terrestrial receptor group bolded.

2.3.1 Terrestrial Vertebrates

Two OPP acceptable studies evaluated acute-oral and 8-day subacute dietary toxicity of formaldehyde on avian species. Avian acute-oral and dietary toxicity data for the northern bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*) categorized formaldehyde as being moderately toxic on an acute oral basis and slightly toxic on a subacute dietary basis (Table 2-10). The median lethal oral dose (LD50) for bobwhite quail was 292.3 mg/kg-bw (Armitage, 1985b) (MRID 00148774). An 8-day dietary study in mallard ducklings (Armitage, 1985a) (MRID 00148775) and bobwhite quail (Armitage, 1985a) (MRID 00148773) reported a median LC50 exceeding 1,850 mg/kg-diet.

Table 2-10. Effects of Formaldehyde in Diet on Avian Species

Test Species	Duration	Endpoint	Hazard Value	Toxicity Category	Citation, MRID[a]
Bobwhite quail	**Acute**	**LD50**	**292.3 mg/kg-bw**	**Moderately toxic**	**(Armitage, 1985b), MRID 00148774**
Bobwhite quail	Subacute (8 days)	LC50	>1,850 mg/kg-diet	Slightly toxic	(Armitage, 1985a), MRID 00148773
Mallard duck	Subacute (8 days)	LC50	>1,850 mg/kg-diet	Slightly toxic	(Armitage, 1985a), MRID 00148775

[a] All listed studies evaluated mortality and are OPP acceptable studies. All OPP studies are identified with MRID. The most sensitive endpoint is bolded.

Four OPP studies, classified as acceptable for quantitative use, summarized mammalian toxicity to formaldehyde and paraformaldehyde through oral routes of exposure (Table 2-11). Technical grade formaldehyde (~37% active ingredient) has moderate toxicity with acute exposure in experimental animals via the oral route. One study excluded from the table below showed no effect to growth at the highest tested concentration of 316 µg/kg/day. The resulting non-determinative endpoint (LOAEC > 0.316 mg/kg/day; MRID 00134114) could not be used quantitatively and was uninformative for hazard characterization.

Table 2-11. Apical Effects of Formaldehyde on Mammals (Oral Exposure)

Test Species	Study Type (% Chemical Purity)	Endpoint(s)	Hazard Value(s) (mg/kg/day) [a]	Effect	Citation, MRID [b]
Dog	**Acute oral – reproductive toxicity (40% formaldehyde)**	**LOAEC; NOAEC**	**3.1; <3.1**	**Pup weight**	**MRID 00143291**
Rat	28-day oral (37% formaldehyde)	LOAEC; NOAEC	279; 808	Weight	MRID 00124677
Rat	90-day oral (95% paraformaldehyde)	LOAEC; NOAEC	95; 48	Weight	(Johannsen et al., 1986)
Dog	90-day oral (95% paraformaldehyde)	LOAEC; NOAEC	95; 71	Weight	(Johannsen et al., 1986)

[a] Endpoints are based on the active ingredient (*e.g.*, formaldehyde, paraformaldehyde)
[b] All listed studies are OPP-acceptable studies.
The most sensitive endpoint identified is bolded.

One OPP study, classified as acceptable for quantitative use, summarized the most sensitive mammal toxicity endpoint to formaldehyde through inhalation exposure (Table 2-12). While inhalation toxicity studies on formaldehyde are extensive, many do not report apical endpoints (mortality, reproduction, growth) which are necessary for ecotoxicity risk evaluation. The most sensitive endpoint that captured effects on an apical endpoint was a 26-week chamber study on rats, hamsters, and monkeys exposed to formaldehyde for 22 hours per day for 26 weeks. Decreased body weights were statistically significant in rats at a concentration of 3.0 ppm from week two (9% decrease) onward (10–15% decrease); however, no differences were observed in hamsters or monkeys. Although this study's formaldehyde exposure duration is longer than the shorter-duration intermittent exposures expected in terrestrial environments from OPP and OPPT uses, the longer duration exposure toxicity endpoints are expected to be protective of those shorter duration exposures.

While OPP does have a screening-level tool to qualitatively estimate avian inhalation toxicity using mammalian toxicity data (Screening Tool for Inhalation Risk, STIR), the data required to conduct this analysis were not available for formaldehyde (acute oral toxicity study using rats: OCSPP guideline study 870.1010, acute inhalation toxicity study using rats: OCSPP guideline study 870.1300). Despite this lack of data, the increased respiration rate of avian species compared to mammals would suggest that avian species would be exposed to higher doses of available airborne formaldehyde and thus inhalation sensitivity to formaldehyde would likely be higher.

Table 2-12. Apical Effects of Formaldehyde on Mammals (Inhalation Exposure)

Test Species	Study Type (% Chemical Purity)	Endpoints	Hazard Values (ppm)	Effect	MRID
Rat	**26-week inhalation (4.96% formaldehyde)**	**LOAEC; NOAEC**	**3.0 (3.68 mg/m^3)** [a] **1.0 (1.23 mg/m^3)** [a]	**Weight**	**MRID 00149755**
[a] Conversion from ppm to mg/m^3 assumes a molecular weight of formaldehyde = 30.031 g/mol.					

The mammalian data come from laboratory data (870 data requirements under 40 CFR 158W) because there were no wildlife ecotoxicity data (40 CRF 158W data requirements 850.2400 on wild mammal toxicity or 850.2500 studies or terrestrial field testing) available. As such, these data are the best available to estimate hazards to mammalian wildlife to formaldehyde. Additionally, these data suggest terrestrial plants are the most sensitive terrestrial receptor group to formaldehyde air exposure using apical endpoints (Table 2-13).

2.3.2 Terrestrial Invertebrates

No acceptable data or high-ranking studies were available to evaluate formaldehyde toxicity to terrestrial invertebrates. For soil invertebrates, because of the volatility and reactivity of formaldehyde in the presence of proteins and nucleic acids (U.S. EPA, 2024a), formaldehyde exposure to terrestrial invertebrates from soil is likely to be minimal. However, air exposure to bees and the direct application of formaldehyde to soil is possible from currently registered OPP uses. The lack of data for terrestrial invertebrate receptor groups may cause uncertainty in risks from these OPP uses.

2.3.3 Terrestrial Plants

Four high-quality studies were identified for evaluating the effects of formaldehyde on terrestrial plants (Table 2-13). No short-term effects were observed in a 4-week fumigation study on the common bean (*Phaseolus vulgaris*) with maximum exposure concentrations of 356 mg/L (438 µg/m^3) (Mutters et al., 1993), although there was a linear increase in growth of shoots beginning at 65 mg/L (78 µg/m^3) formaldehyde exposure (Mutters et al., 1993). Reduced growth of pollen tube lengths of lily plants (*Lilium longiflorum*) has also been measured with acute formaldehyde exposure with inhibition of pollen tube growth at 450 µg/m^3 with 5 hours of exposure (72.5% reduction in pollen tube length) and at 1,720 µg/m^3 with 1 hour of exposure through fumigation (13.5% reduction in pollen tube length) (Masaru et al., 1976). In *Bromeliaceae* plants (epiphytes), 12 hours of exposure to formaldehyde vapor in chamber experiments at a concentration of 1,000 µg/m^3 reduced chlorophyll content by 17.3 percent (Li et al., 2014). It should be noted that while this study demonstrated an adverse effect, chlorophyll content is not an apical toxicity endpoint (mortality, growth, reproduction), and therefore can only be used qualitatively for ecological hazard characterization.

In a controlled experiment exposing plants to formaldehyde in fog water periodically over 8 months at nominal low (100 µM), medium (500 µM), and high (1,000 µM) concentrations, Douglas fir (*Pseudotsuga menziesii*) height and diameter decreased relative to controls at the lowest treatment concentration of 91.57 μM (3,300 μg/m^3). No effects on lichen (*Lobaria pulmonaria*) growth were observed in this study, even at the highest concentration of 948.67 μM (34,188 μg/m^3) (Muir and Shirazi, 1996).

Table 2-13. Effects of Formaldehyde in Air on Terrestrial Plants

Test Species	Duration	Endpoint	Hazard Value (μg/m^3)	Effect	Citation, MRID[a]
Common bean	**4 weeks**	**NOAEC**	**438**	**N/A**	**(Mutters et al., 1993)**
Lily	5 hours	LOAEC	450	Growth	(Masaru et al., 1976)
Bromeliaceae	12 hours	LOAEC	1,000	Chlorophyll	(Li et al., 2014)
Douglas fir	8 months	LOAEC	3,300	Growth	(Muir and Shirazi, 1996)
Lichen	8 months	NOAEC	34,188	Growth	(Muir and Shirazi, 1996)

[a] High-ranking studies from OPPT systematic review.
The most sensitive endpoint identified is bolded.

2.3.4 Summary

Formaldehyde may exist in various forms when released to aquatic environments (U.S. EPA, 2024a). Thus, EPA examined ecological effects data and information on the chemical properties of formaldehyde, methylene glycol, and paraformaldehyde. Despite unreliable ECOSAR predictions for methylene glycol, similarities in chemical structure and properties, as well as data on paraformaldehyde toxicity, supported the Agency assumption that formaldehyde toxicity is representative and protective of toxicity to paraformaldehyde and methylene glycol and could be used to represent toxicity to these various forms of formaldehyde in solution.

The most sensitive ecotoxicity endpoints from each receptor group (Table ES-1) and OPP ecotoxicity categories suggest formaldehyde is moderately toxic to birds and mammals via diet, moderately toxic to freshwater fish, moderately toxic to marine fish, moderately toxic to marine invertebrates, and highly toxic to freshwater invertebrates on an acute basis (U.S. EPA, 2008). Toxicity with chronic exposure was generally an order of magnitude lower (*i.e.*, more toxic) than toxicity with acute exposure for freshwater fish and freshwater invertebrates. Reliable and protective chronic exposure toxicity data were lacking for aquatic invertebrates. EPA therefore used an ACR to estimate chronic exposure toxicity to the most acutely sensitive freshwater invertebrate (*i.e.*, ostracods). The calculated chronic NOAEC for ostracods to formaldehyde using this ACR was 0.063 mg FDH/L.

It should be noted that most studies presented here used formalin (solution of water, 37% formaldehyde, and often 6–15% methanol) as the test substance for dose-response toxicity tests. Use of this solution for toxicity tests results in some uncertainty in whether toxicity is directly related to formaldehyde alone or the combination of methanol and formaldehyde. However, all ecotoxicity endpoints reported in this assessment are adjusted to represent toxicity to formaldehyde alone.

Reliable high-quality data were not available for terrestrial invertebrates (*e.g.*, honeybees) or nonvascular plants. Given the current lack of data for these receptor groups, if exposure is expected, risk will be assumed. However, ECOSAR predictions of formaldehyde toxicity to aquatic receptor groups may be good estimations for risk characterization until data are available.

EPA/OPPT uses several considerations when weighing and weighting 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

(Table_Apx A-4). This approach is consistent with the 2021 Draft Systematic Review Protocol Supporting TSCA Risk Evaluations (U.S. EPA, 2021). Table_Apx A-4 summarizes how these considerations were ranked for each environmental hazard receptor. Overall, EPA/OPPT considers the evidence for aquatic acute fish toxicity to be robust, the evidence for aquatic chronic fish toxicity and acute invertebrate toxicity to be moderate, and the evidence for aquatic plant toxicity to be slight. For terrestrial receptors, the evidence for vertebrates and plants was slight and the evidence for invertebrates was indeterminate.

REFERENCES

Armitage, TM. (1985a). R2002280: DER for (Fletcher, 1983). (MRID 00148775; MRID 00148773). Armitage, TM.

Armitage, TM. (1985b). R2002282: DER for (Fletcher, 1984). (MRID 00148774). Armitage, TM.

Bills, TD; Chandler, JH, Jr.; Marking, LL. (1977). Formalin: Its toxicity to nontarget aquatic organisms, persistence, and counteraction. In Investigations in Fish Control (pp. 1-7). (Investigations in Fish Control 73). Washington, DC: U.S. Fish and Wildlife Service. https://pubs.er.usgs.gov/publication/ifc73

Brooke, L. (1987). Report of the flow-through and static acute test comparisons with fathead minnows and acute tests with an amphipod and a cladoceran (pp. 24 p.). Superior, WI: Center for Lake Superior Environmental Studies, University of Wisconsin.

Burnham, KP; Anderson, DR. (2002). Model selection and multimodel inference: a practical information-theoretic approach (2nd ed.). New York: Springer. http://www.springer.com/statistics/statistical+theory+and+methods/book/978-0-387-95364-9

Cook, NJ. (1975). R2003028: DER for (Heitmuller, 1975). (MRID 00126395). Cook, NJ.

Edmundson, JP, Jr. (1975). R2003027: DER for (Bentley, 1975). (MRID 00101857; MRID 00101865). Edmundson, JP Jr.

Fajer-Avila, EJ; Abdo-De la Parra, I; Aguilar-Zarate, G; Contreras-Arce, R; Zaldivar-Ramirez, J; Betancourt-Lozano, M. (2003). Toxicity of formalin to bullseye puffer fish (Sphoeroides annulatus Jenyns, 1843) and its effectiveness to control ectoparasites. Aquaculture 223: 41-50. http://dx.doi.org/10.1016/S0044-8486(03)00166-2

Howe, GE; Marking, LL; Bills, TD; Schreier, TM. (1995). Efficacy and toxicity of formalin solutions containing paraformaldehyde for fish and egg treatments. Prog Fish Cult 57: 147-152. http://dx.doi.org/10.1577/1548-8640(1995)057<0147:EATOFS>2.3.CO;2

Institut, F. (2008). [Redacted] Daphnia magna reproduction test of formaldehyde according to Guideline OECD 211. (IF-08/01232312). Ludwigshafen, Germany: BASF SE.

Johannsen, FR; Levinskas, GJ; Tegeris, AS. (1986). Effects of formaldehyde in the rat and dog following oral exposure. Toxicol Lett 30: 1-6. http://dx.doi.org/10.1016/0378-4274(86)90171-2

King, K; Farrell, P. (2002). Sensitivity of juvenile Atlantic sturgeon to three therapeutic chemicals used in aquaculture. N Am J Aquac 64: 60-65. http://dx.doi.org/10.1577/1548-8454(2002)064<0060:SOJAST>2.0.CO;2

Li, P; Pemberton, R; Zheng, G. (2014). Foliar trichome-aided formaldehyde uptake in the epiphytic Tillandsia velutina and its response to formaldehyde pollution. Chemosphere 119C: 662-667. http://dx.doi.org/10.1016/j.chemosphere.2014.07.079

Masaru, N; Syozo, F; Saburo, K. (1976). Effects of exposure to various injurious gases on germination of lily pollen. Environ Pollut 11: 181-187. http://dx.doi.org/10.1016/0013-9327(76)90082-3

Muir, PS; Shirazi, AM. (1996). Effects of formaldehyde-enriched mists on Pseudotsuga menziesii (Mirbel) Franco and Lobaria pulmonaria (L.) Hoffm. Environ Pollut 94: 227-234. http://dx.doi.org/10.1016/S0269-7491(96)00054-1

Mutters, RG; Madore, M; Bytnerowicz, A. (1993). Formaldehyde exposure affects growth and metabolism of common bean. J Air Waste Manag Assoc 43: 113-116. http://dx.doi.org/10.1080/1073161X.1993.10467112

Natella, CM. (1975). R2002346: DER for (Smith, 1985). (MRID 00148772). Natella, CM.

Omoregie, E; Ofojekwu, PC; Amali, EI. (1998). Effects of sublethal concentrations of formalin on weight gain in the Nile tilapia, Oreochromis niloticus (Trewavas). Asian Fisheries Science 10: 323-327.

Singh, BB; Chandra, R; Sharma, YK. (2008). Effect of pyridine and formaldehyde on a macrophyte (Lemna minor L.) and a sludge worm (Tubifex tubifex Muller) in fresh water microcosms. Applied Ecology and Environmental Research 6: 21-35.

Takayanagi, K; Sakami, T; Shiraishi, M; Yokoyama, H. (2000). Acute toxicity of formaldehyde to the pearl oyster Pinctada fucata martensii. Water Res 34: 93-98. http://dx.doi.org/10.1016/S0043-1354(99)00101-3

U.S. EPA. (2008). Technical overview of ecological risk assessment analysis phase: Ecological effects characterization [Website]. http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm

U.S. EPA. (2011). Evaluation guidelines for ecological toxicity data in the open literature. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/evaluation-guidelines-ecological-toxicity-data-open#guidance

U.S. EPA. (2021). Draft systematic review protocol supporting TSCA risk evaluations for chemical substances, Version 1.0: A generic TSCA systematic review protocol with chemical-specific methodologies. (EPA Document #EPA-D-20-031). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.regulations.gov/document/EPA-HQ-OPPT-2021-0414-0005

U.S. EPA. (2024a). Chemistry, Fate, and Transport Assessment for Formaldehyde. Washington, DC: U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics.

U.S. EPA. (2024b). Risk Evaluation for Formaldehyde: Systematic review supplemental file: Data quality evaluation information for environmental hazard. Washington, DC: Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention.

MRID 00028002. Wellborn, T.L., Jr. (1969) The Toxicity of Nine Therapeutic and Herbicidal Compounds to Striped Bass. Progressive Fish-Culturalist 31: 27-32. (Also in Unpublished Submission Received August 20, 1976, under 39445-1; Submitted by American Carbonyl, Inc., Tenafly, NJ. CDL: 228232-C, Fiche/Master ID #00028002.

MRIDs 00065640, 00082150. Birdsong, C.L.; Avault, J.W. Jr. 1971. Toxicity of certain chemicals to juvenile pompano. Progressive-Fish Culturist 33:76-80. (Available from: Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402; published study; CDL: 242555-N).

MRIDs 00101857, 00101865. Bentley, R. (1975) Acute Toxicity of Ten Baroid Compounds to Bluegill and Rainbow Trout (Salmo gairdneri). (Unpublished Study Received May 20, 1975 under 17664-8; prepared by Bionomics, EG & G, submitted by Baroid, Div. of N. L. Industries, Inc., Houston, TX; CDL: 222885-A)

MRID 00124738. Wilford, W.A. (1966) Toxicity of 22 Therapeutic compounds to six fishes. Investigations in Fish Control, No. 18. USM Bur Sport Fish and Wildlife, Res. Publ. 35 10pp. Acc. No. 2516811.

MRID 00126395. Heitmuller, T. (1975) Acute Toxicity of Aldecide to Larvae of the Easter Oyster (Crassostrea virginica), Pink Shrimp (Penaeus duorarum), and Fiddler Crabs (Uca pugilator). (Unpublished Study Received July 15, 1975, Under 17664-8; Prepared by Bionomics EG & G, Inc., Submitted by Baroid Div., N.L. Industries, Inc., Houston, TX. CDL: 222881-A).

MRIDs 00126396, 00128086. Heitmuller, T. (1975) Acute Toxicity of Surflo-B17 to Larvae of the Easter Oyster (Crassostrea virginica), Pink Shrimp (Penaeus duorarum), and Fiddler Crabs (Uca pugilator). (Unpublished Study Received July 15, 1975, Under 17664-5; Prepared by Bionomics EG & G, Inc., Submitted by Baroid Div., N.L. Industries, Inc., Houston, TX. CDL:222880-). Fiche/Master ID 00126394.

MRID 00132485. Bills, T.D., L.L. Marking and J.H. Chandler, Jr. (1977). Formalin: Its toxicity to nontarget aquatic organism, persistence. and counteraction. U.S. Fish & Wildlife Service. Invest. Fish Control 73:l-7. Acc. No. 2516811.

MRID 00134123. McCann, J.; Pitcher, F. 1973. Russell's Incubator Fumigant: Bluegill: Test No. 577. (Unpublished study received May 13, 1973 under 346-14; prepared by Pesticides Regulation

Div., Animal Biology Laboratory, submitted by U.S. Environmental Protection Agency, Beltsville, MD; CDL: 128358-A).

MRID 00143291. Hurni, H., Ohder, H. (1973) Reproduction study with formaldehyde and hexamethylenetetramine in beagle dogs. Food and Cosmetics Toxicology. 11: 459-462. https://doi.org/10.1016/0015-6264(73)90010-2

MRID 00148770. Lemon, K.A. (1985) Acute Toxicological Evaluations of Estuarine Organisms to Support Registration Action 44797-RL. Prepared by Envirosystems, Incorporated, Hampton, NH. Submitted by N. L. Treating Chemical Company, Houston, TX. Accession Nos. 257124 and 257185.

MRID 00148772. Smith, E.H. (1985) Acute Toxicity of Formalin on Daphnia magna to Support Registration Action 44797-RL (and 44797-RA). Prepared by ANATEC Laboratories , Inc., 435 Tesconi Circle, Santa Rosa, CA 95401. Submitted by N.L. Treating Chemicals, Houston, TX. Accession Nos. 257124 and 257125.

MRID 00148773. Fletcher, Dale W. (1983) Report to NL Treating Chemicals, NL Industries, Inc., 8-Day Dietary LC50 Study with Surflo-B315 in Mallard Ducklings. Prepared by Bio-Life Associates, Ltd., Neillsville, WI.

MRID 00148774. Fletcher, Dale W. (1984) Report to NL Treating Chemicals, NL Industries, Inc., Acute Oral Toxicity with Surflo-B315 in Bobwhite Quail. Prepared by Bio-Life Associates, Ltd., Neillsville, WI . BLAL No 83 QD 38.

MRID 00148775. Fletcher, Dale W. (1983) Report to NL Treating Chemicals, NL Industries, Inc., 8-Day Dietary LC50 Study with Surflo-B315 in Bobwhite Quail. Prepared by Bio-Life Associates, Ltd., Neillsville, WI .

MRID 00149755. Clary, J. (1980) A 26 Week Inhalation Study of Formaldehyde in the Monkey, Rat, and Hamster. Prepared by Bio/dynamics Inc. Division of Biology and Safety Evaluation. Project No. 79-7259. Submitted by: Formaldehyde Institute, 1075 Central Park Ave., Scardale, NY.

APPENDICES

Appendix A ENVIRONMENTAL HAZARD DETAILS

A.1 OPP Ecotoxicity Categories

Table_Apx A-1. Ecotoxicity Categories for Terrestrial and Aquatic Organisms

Toxicity Category	Avian: Acute Oral Concentration (mg/kg-bw)	Avian: Dietary Concentration (mg/kg-diet)	Aquatic Organisms: Acute Concentration (mg/L)	Wild Mammals: Acute Oral Concentration (mg/kg-bw)	Non-Target Insects: Acute Concentration (µg/bee)
Very highly toxic	<10	<50	<0.1	<10	–
Highly toxic	10–50	50–500	0.1–1	10–50	<2
Moderately toxic	51–500	501–1,000	>1–10	51–500	2–11
Slightly toxic	501–2000	1,001–5,000	>10–100	501–2,000	–
Practically nontoxic	>2,000	>5,000	>100	>2,000	>11

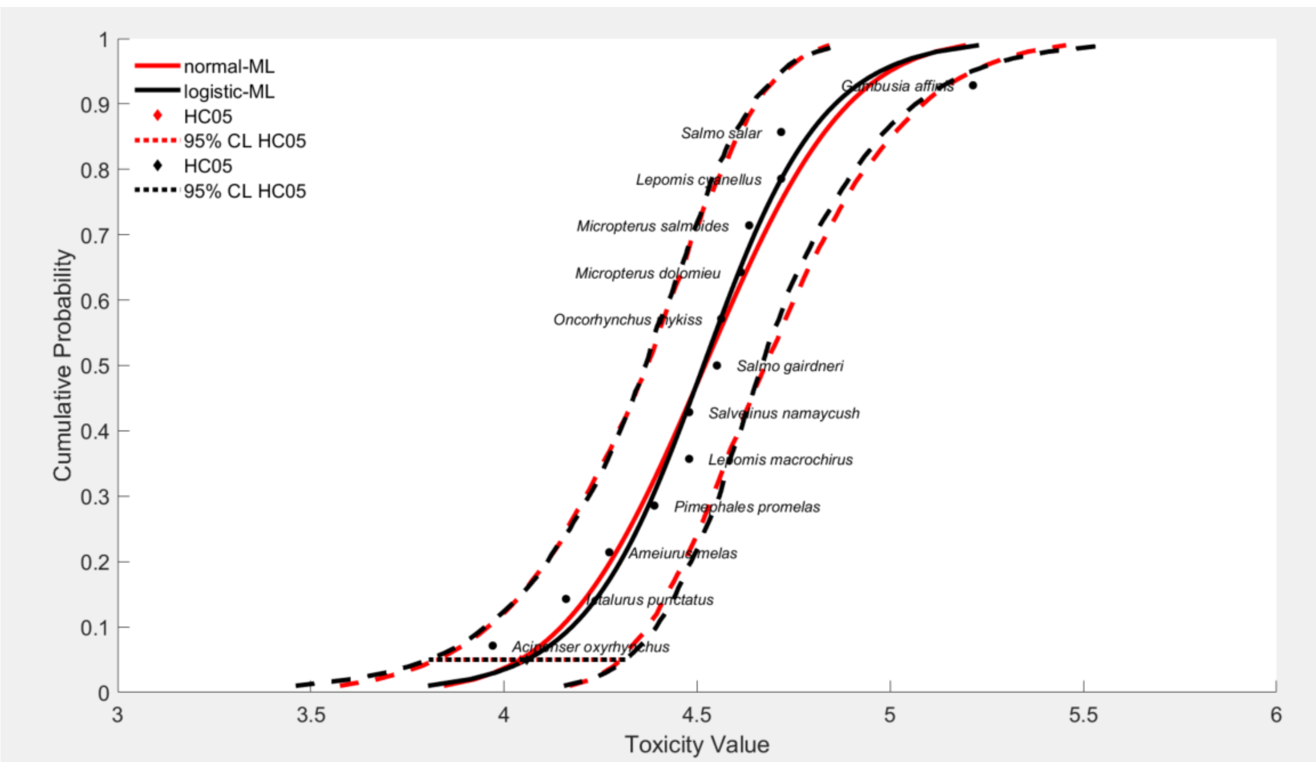
A.2 Species Sensitivity Distribution (SSD)

The SSD Toolbox 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 formaldehyde risk evaluation, EPA created one SSD with the SSD Toolbox to evaluate acute fish 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 fish were curated to prioritize study quality and to assure comparability between toxicity values. For example, the dataset included only LC50s for 96-hour assays that measured mortality for aquatic vertebrates. Table_Apx A-2 shows the data that were used in the SSD. With this dataset, the SSD Toolbox was used to apply a variety of algorithms to fit and visualize SSDs with different distributions. Table_Apx A-2 shows the SSD Toolbox interface after each distribution and fitting method was fit to the data. An HC05 is calculated for each (Table_Apx A-3).

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 (AIC_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 AIC_c value, and therefore the best fit for the data was the logistic model (Figure_Apx A-3). The results for this model predicted 5 percent of the species (HC05) to have their LC50s exceeded at 11.47 mg/L (7.96–14.97 mg/L 95% CI). The HC50 was estimated at 32.84 mg/L (23.59–45.72 mg/L 95% CI) and the HC95 was estimated 94.04 mg/L (52.38–168.84 mg/L 95% CI).

Table_Apx A-2. Species Sensitivity Distribution (SSD) Model Input for Acute Exposure Toxicity in Freshwater Fish

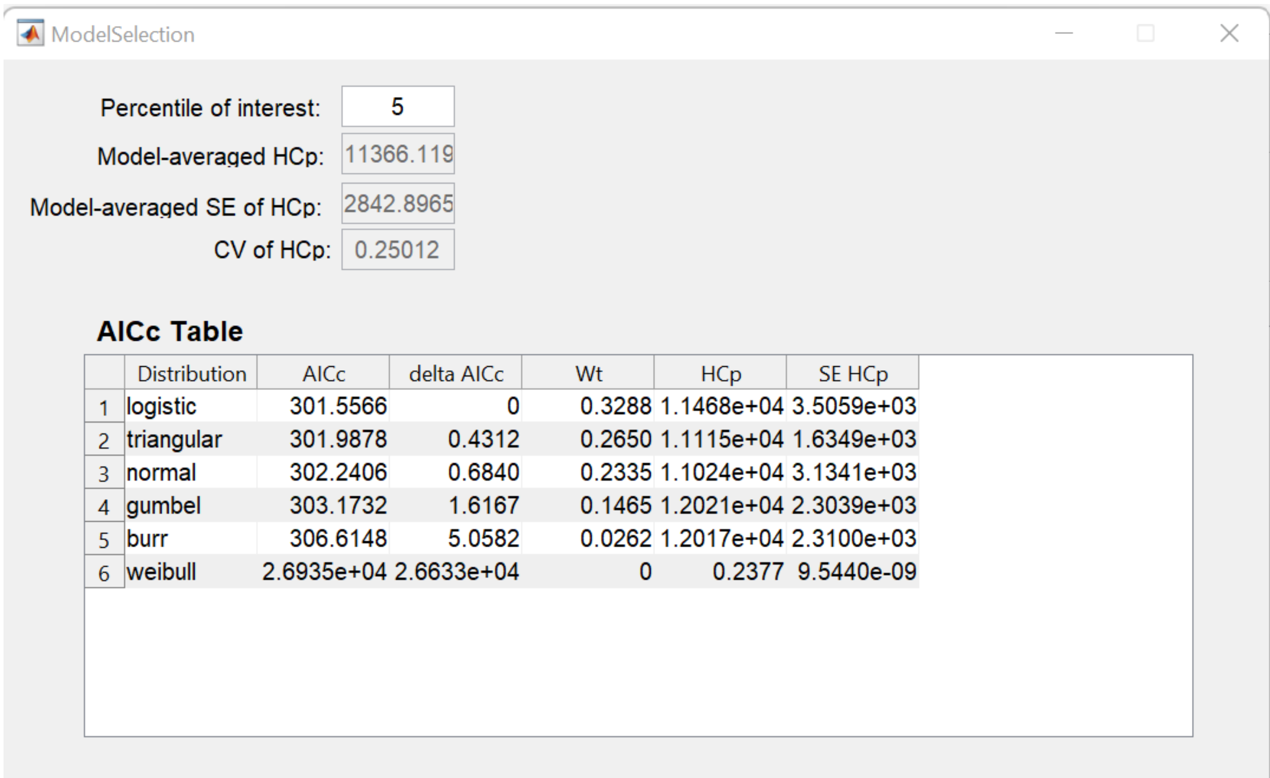
Genus	Species	Acute Toxicity Value 96-Hour LC50s (µg/L)
Acipenser	*oxyrhynchus*	9,348
Ictalurus	*punctatus*	10,554
Ameiurus	*melas*	18,726
Ictalurus	*punctatus*	19,842
Pimephales	*promelas*	24,500
Lepomis	*macrochirus*	30,155
Salvelinus	*namaycush*	30,155
Salmo	*gairdneri*	35,583
Onchorhynchus	*mykiss*	36,488
Micropterus	*dolomieu*	41,011
Micropterus	*salmoides*	43,122
Salmo	*salar*	52,168
Lepomis	*cyanellus*	52,168
Gambusia	*affinis*	163,700



Figure_Apx A-1. Species Sensitivity Distribution (SSD) for Acute Exposure Toxicity to Aquatic Vertebrates (Fish)

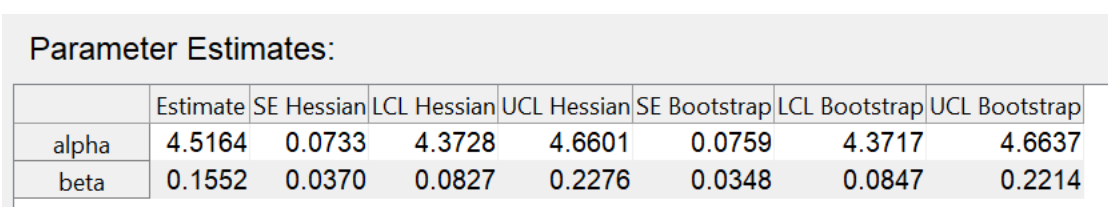
Table_Apx A-3. SSD Model Predictions for Acute Exposure Toxicity to Aquatic Vertebrates (Fish) Using the Maximum Likelihood Method

Distribution	HC05 (µg/L)	P value
Normal	11,204	0.7742
Logistic	**11,468**	**0.8152**
Triangular	11,115	0.5295
Gumbel	12,021	0.3407
Weibull	2,377	0.000999
Burr	12,017	0.3147
The model with the lowest AIC_c value, and therefore best fit, is bolded.		



	Distribution	AICc	delta AICc	Wt	HCp	SE HCp
1	logistic	301.5566	0	0.3288	1.1468e+04	3.5059e+03
2	triangular	301.9878	0.4312	0.2650	1.1115e+04	1.6349e+03
3	normal	302.2406	0.6840	0.2335	1.1024e+04	3.1341e+03
4	gumbel	303.1732	1.6167	0.1465	1.2021e+04	2.3039e+03
5	burr	306.6148	5.0582	0.0262	1.2017e+04	2.3100e+03
6	weibull	2.6935e+04	2.6633e+04	0	0.2377	9.5440e-09

Figure_Apx A-2. SSD Toolbox Model Fit Parameters

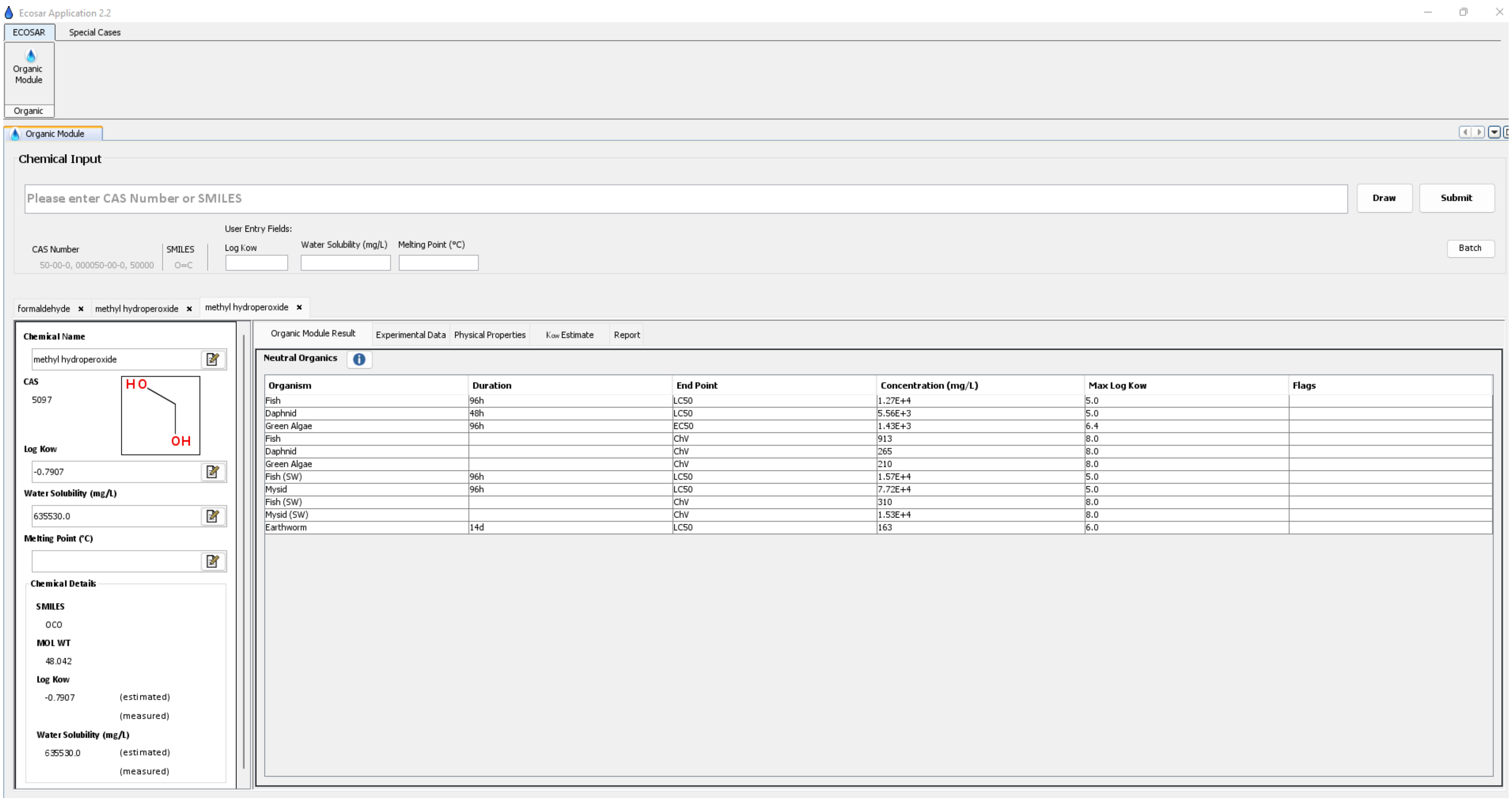


	Estimate	SE Hessian	LCL Hessian	UCL Hessian	SE Bootstrap	LCL Bootstrap	UCL Bootstrap
alpha	4.5164	0.0733	4.3728	4.6601	0.0759	4.3717	4.6637
beta	0.1552	0.0370	0.0827	0.2276	0.0348	0.0847	0.2214

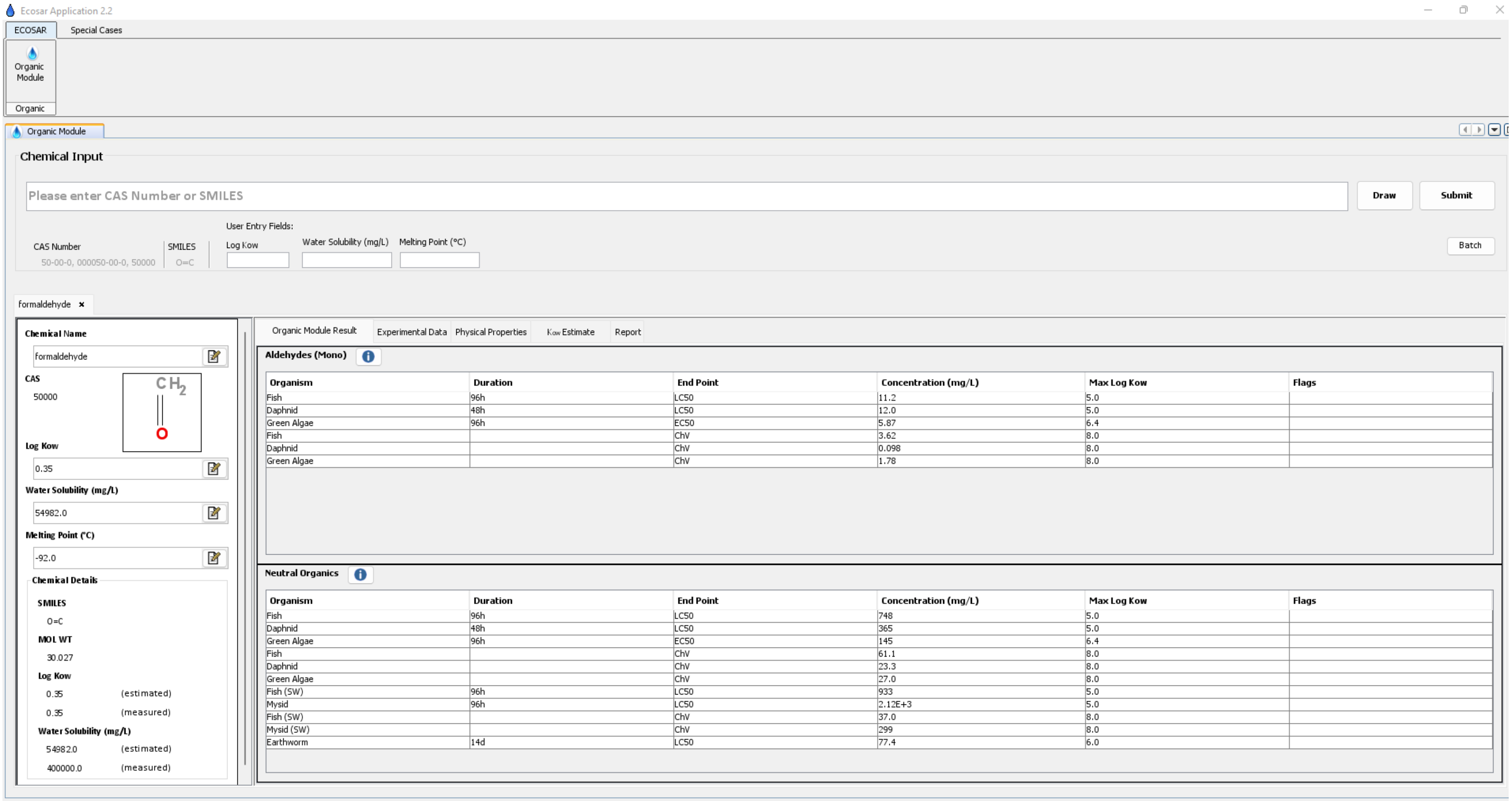
Figure_Apx A-3. Parameter Estimates for the Selected Logistic Model Using the Maximum Likelihood Method

A.3 Ecological Structure Activity Relationship (ECOSAR) Predictions

The ECOSAR Class Program is a predictive model that estimates aquatic toxicity by grouping structurally similar chemicals. ECOSAR was developed and is maintained by the EPA for screening-level assessments to evaluate aquatic hazard in the absence of quality experimental data. ECOSAR predictions were used to evaluate aquatic toxicity of formaldehyde and methylene glycol.



Figure_Apx A-4. ECOSAR Inputs and Outputs for Methylene Glycol (SMILES Code: C(O)C)



Figure_Apx A-5. ECOSAR Inputs and Outputs for Formaldehyde

A.4 Weight of Scientific Evidence

Table_Apx A-4. Evidence Table Summarizing the Overall Confidence Derived from Hazard Thresholds

Types of Evidence	Quality of the Database	Consistency	Strength and Precision	Biological Gradient/ Dose-Response	Relevance	Hazard Confidence
Aquatic						
Acute Aquatic Vertebrate Assessment	+++	+++	+++	+++	+++	Robust
Acute Aquatic Invertebrate Assessment	++	+	++	++	+++	Moderate
Chronic Aquatic Assessment	++	+	+	+	++	Moderate
Aquatic Plant Assessment	+	+	+	+	+++	Slight
Terrestrial						
Terrestrial Plants	++	++	++	++	+++	Moderate
Terrestrial Vertebrates	++	+	+	+	++	Slight
Terrestrial Invertebrates	+	+	+	+	+	Indeterminate[b]

[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.

[b] Indeterminate is assigned when there is no available data for which to evaluate potential hazard.

EPA used the strength-of-evidence and uncertainties from Table_Apx A-4 for the hazard characterization to qualitatively rank the overall confidence using evidence for environmental hazard. Confidence levels of robust (+ + +), moderate (+ +), slight (+), or indeterminant are assigned for each evidence property that corresponds to the evidence considerations described in Table_Apx A-4. The rank of the *Quality of the Database* consideration is based on the systematic review data quality rank (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 ranks correspond to the evidence table ranks of robust (+ + +), moderate (+ +), or slight (+), respectively. The evidence considerations are weighted based on professional judgement 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 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.
- Indeterminant (N/A) corresponds to entries in evidence tables where information is not available within a specific evidence consideration.

Types of Uncertainties

The 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 (Table 2-13).

- 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.

The evidence table 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, although de-emphasizing an individual ranking that may give the impression that ranks are cumulative (*e.g.*, ranks of different categories may have different weights).