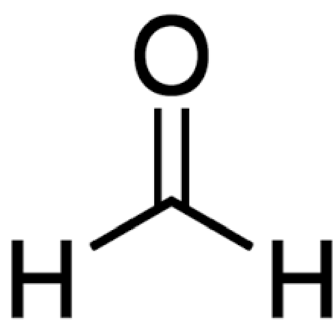
**United States**
**Environmental Protection Agency**

**March 2024**
**Office of Chemical Safety and Pollution Prevention**

# Draft Environmental Hazard Assessment for Formaldehyde

## CASRN 50-00-0



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**Docket**

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**Key Points: Environmental Hazards for Formaldehyde**

EPA considered all reasonably available information identified by the Agency through its systematic review process under Toxic Substances Control Act (TSCA) and submissions under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to characterize formaldehyde environmental hazard. The bullets below summarize the key points of this draft assessment.

- Formaldehyde is known to readily convert to methylene glycol, various oligomers, and paraformaldehyde; EPA determined that formaldehyde toxicity data are protective or capture the toxicity of these compounds.
- A high-quality dataset for aquatic organisms indicated the following:
  - Based on acute exposure and mortality endpoints (24- to 96-hour lethal concentrations at which 50 percent of test organisms die [LC50s]), formaldehyde is slightly to moderately toxic to aquatic vertebrates with the most sensitive endpoint identified in marine/estuarine fish (2.92 mg/L).
  - Formaldehyde acute exposure toxicity to freshwater invertebrates is variable depending on species and ranges from practically nontoxic to highly toxic with the most sensitive endpoint identified in ostracods (0.32 mg/L; 96-hour LC50, immobility).
  - Formaldehyde chronic exposure toxicity to freshwater fish and invertebrates are approximately an order of magnitude more toxic than acute exposure toxicity.
- High-quality data were limited across terrestrial organisms; however, available data indicated:
  - Formaldehyde is slightly to moderately toxic to birds via diet and moderately toxic to mammals via the oral routes of exposure.

# 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 draft 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 to 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, 2024). 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 (mg/L) [a b] | Toxicity Category | Citation or MRID |
|---|---|---|---|---|
| Freshwater fish | Acute | LC50 = 9.35 | Moderately toxic | (Fajer-Avila et al., 2003) |
| | Chronic | NOAEC = 0.62 (21% reduction in weight gain, $P > 0.05$)<br>LOAEC = 1.25 (40% reduction in weight gain) | N/A | (Omoregie et al., 1998) |
| Freshwater invertebrates | Acute | LC50 = 0.32 | Highly toxic | (Bills et al., 1977), MRID 00132485 |
| | Chronic | 0.063 | N/A | ACR[b] |
| Freshwater vascular plants | N/A | EC50 = 0.18 (biomass)<br>LOAEC = 0.1 (25% reduction in biomass)<br>NOAEC < 0.1 | 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 | Moderately toxic | (Takayanagi et al., 2000) |
| | Chronic | No data | N/A | N/A |
| Estuarine/marine invertebrates | Acute | LC50 = 1.96 | 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 |

| Receptor Group | Exposure Scenario | Toxicity Endpoint (mg/L) [a b] | Toxicity Category | Citation or MRID |
|---|---|---|---|---|
| | Subacute dietary | LC50 > 1,850 mg/kg-diet | Slightly toxic | MRID 00148775 |
| Mammals | Acute oral | LOAEC = 3.1 mg/kg/day<br>NOAEC < 3.1<br>(based on pup weight) | Moderately toxic | MRID 00143291 |
| | 26-week inhalation | LOAEC = 3.0 ppm (3.68 $mg/m^3$)<br>NOAEC = 1.0 ppm (1.23 $mg/m^3$) | N/A | MRID 00149755 |
| Terrestrial plants | N/A | 438 $\mu g/m^3$<br>(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] An acute-to-chronic ratio (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.

# 1 INTRODUCTION

This draft 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

This 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 draft environmental hazard assessment is shaded blue. In some cases, modular assessments were completed jointly under TSCA and FIFRA. These modules are shown in dark gray.

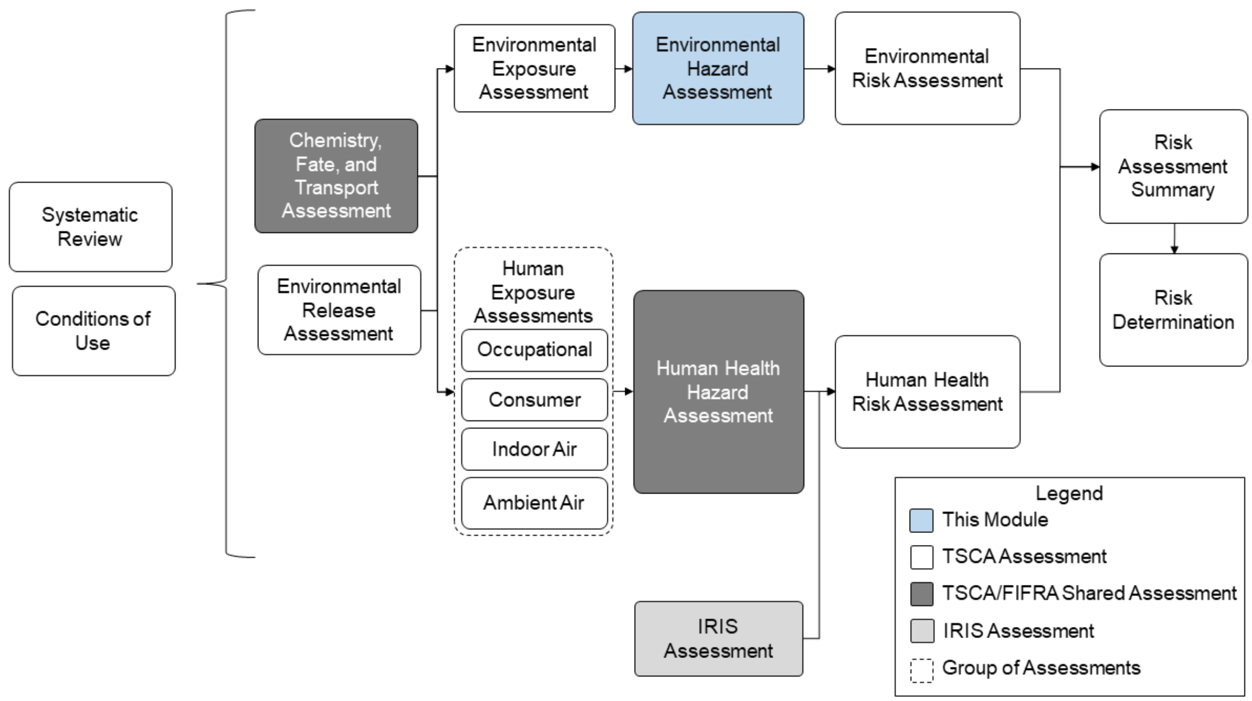


**Figure 1-1. Risk Evaluation Document Summary Map**

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

## 1.2 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 (*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, 2023).

- ***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 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) (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 (mg/L) [b] | Citation, MRID [c] |
|---|---|---|---|---|
| Formaldehyde | 37 | Rainbow trout | 35.6<br>36.5<br>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 |

| Compound | Chemical Purity (%) [a] | Test Species | Hazard Value (mg/L) [b] | Citation, MRID [c] |
|---|---|---|---|---|
| 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 (*e.g.*, (Golden and Valentini, 2014).

**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 |

| 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 |
|---|---|---|---|---|---|
| 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 draft 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*) (McCorkle et al., 1979) 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 to 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) |
| 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 |

| Test Species | Duration (hours) | Endpoint | Chemical Purity (%) [a] | Hazard Value (mg/L) [b] | Toxicity Category | Citation, MRID[c] |
|---|---|---|---|---|---|---|
| Green sunfish | 96 | LC50 | 37 | 52.17 | Slightly toxic | (Bills et al., 1977), MRID 00132485 |
| Mosquitofish | 96 | LC50 | 100 | 163.70 | Practically nontoxic | (McCorkle et al., 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 |

| Test Species | Duration (hours) | Endpoint | Chemical Purity (%) [a] | Hazard Value (mg/L) [b] | Toxicity Category | Citation, MRID[c] |
|---|---|---|---|---|---|---|
| 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 the water flea (*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 to 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 | Chemical Purity (%) [a] | Hazard Value (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) |

| Test Species | Duration (days) | Endpoint | Chemical Purity (%) [a] | Hazard Value (mg/L) [b] | Effect | Citation [c] |
|---|---|---|---|---|---|---|
| Sludge worm | 21 | LC50 | 100 | 0.39 | Mortality | (Singh et al., 2008) |
| Sludge worm | 21 | NOAEC<br>LOAEC | 100 | <0.1<br>0.1 | Growth | (Singh et al., 2008) |
| Water flea | 21 | EC50 | 40 | 9.6 | Reproduction | (Institut, 2008) |
| Water flea | 21 | NOAEC<br>LOAEC | 40 | 1.04<br>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** | |

| Test Species | Duration | Endpoint | Chemical Purity (%) [a] | Hazard Value (mg/L) [b] | Effect | Citation [c] |
|---|---|---|---|---|---|---|
| | | **LOAEC** | **100** | **0.1** | **Biomass (25% reduction)** | **(Singh et al., 2008)** |
| | | **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)** |

| Test Species | Duration (hours) | Endpoint | Chemical Purity (%) [a] | Hazard Value (mg/L) [b] | Toxicity Category | Citation[c] |
|---|---|---|---|---|---|---|

[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, 2024). 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 $mg/m^3$. 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** |

| Test Species | Duration | Endpoint | Hazard Value | Toxicity Category | Citation, MRID[a] |
|---|---|---|---|---|---|
| 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 (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 to 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) | Endpoint(s) | Hazard Value (ppm) | Effect | MRID |
|---|---|---|---|---|---|
| **Rat** | **26-week Inhalation (4.96% formaldehyde)** | **LOAEC; NOAEC** | **3.0 (3.68 mg/m$^3$) [a]<br>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, 2024), formaldehyde exposure to terrestrial invertebrates from soil is likely to be minimal. As such, additional toxicity data for formaldehyde exposure to soil invertebrates is not anticipated to be needed for OPPT or OPP risk evaluations of formaldehyde.

### 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 mg/m3) (Mutters et al., 1993), although there was a linear increase in growth of shoots beginning at 65 mg/L (78 mg/m3) 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 1,400 mg/L (1680 mg/m$^3$) in a 5-hour fumigation experiment (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 ($\mu g/m^3$) | Effect | Citation, MRID[a] |
|---|---|---|---|---|---|
| **Common bean** | **4 weeks** | **NOAEC** | **438** | **N/A** | **(Mutters et al., 1993)** |
| Lily | 5 hours | LOAEC | 1,680 | 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.

## 2.4 Summary

Formaldehyde may exist in various forms when released to aquatic environments (U.S. EPA, 2024). 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 ratio 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 to 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. Ecotoxicity data for these receptor groups are not anticipated to be needed for future formaldehyde risk evaluations. 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 *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances (*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.

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# 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 |

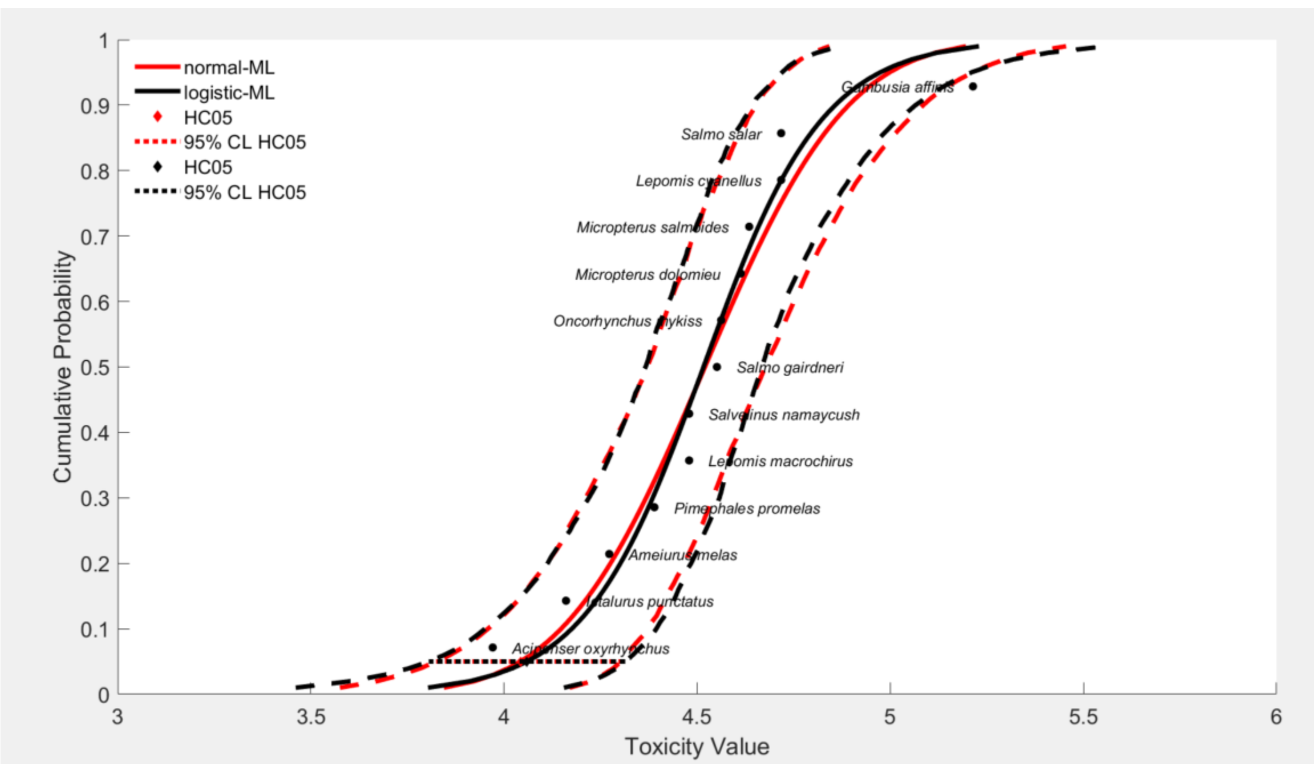
## A.2 Species Sensitivity Distribution (SSD)

The SSD Toolbox is a resource created by EPA's Office of Research and Development (ORD) that can fit SSDs to environmental hazard data (U.S. EPA, 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**

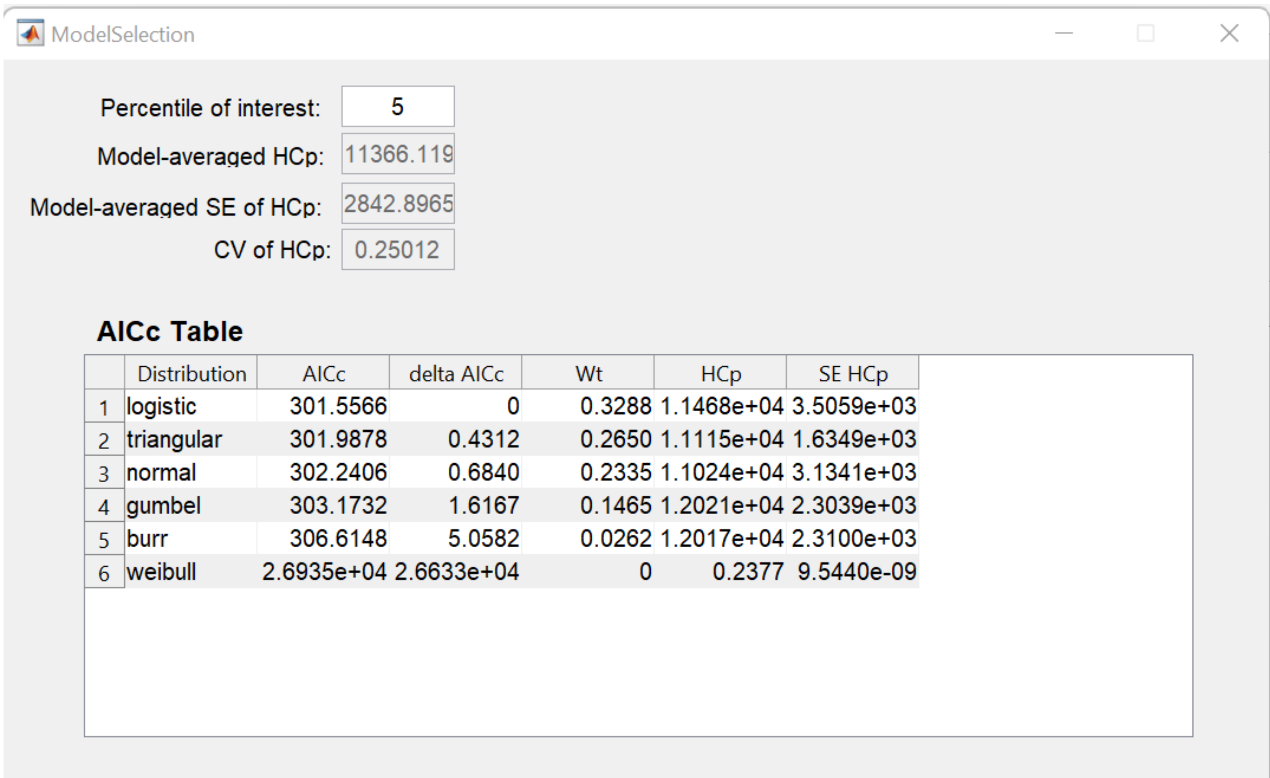
| Genus | Species | Acute Toxicity Value 96-hour LC50s (µg/L) |
|---|---|---|
| *Acipenser* | *oxyrhynchus* | 9,348 |
| *Ictalurus* | *punctatus* | 10,554 |
| *Ameiurus* | *melas* | 18,726 |
| *Ictalurus* | *punctatus* | 19,842 |
| *Pimephales* | *promelas* | 24,500 |
| *Lepomis* | *macrochirus* | 30,155 |
| *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)**

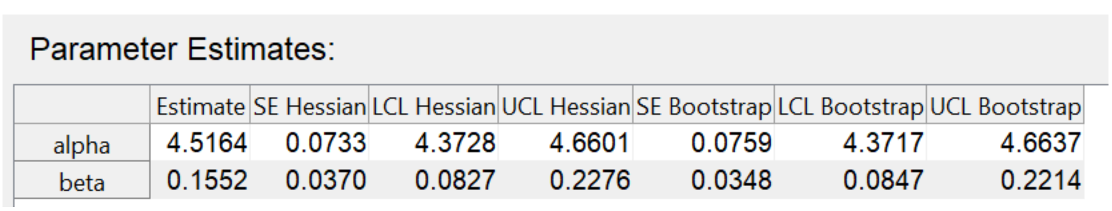
**Table_Apx A-3. SSD Model Predictions for Acute Exposure Toxicity to Aquatic Vertebrates (Fish) Using the Maximum Likelihood Method**

| 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**

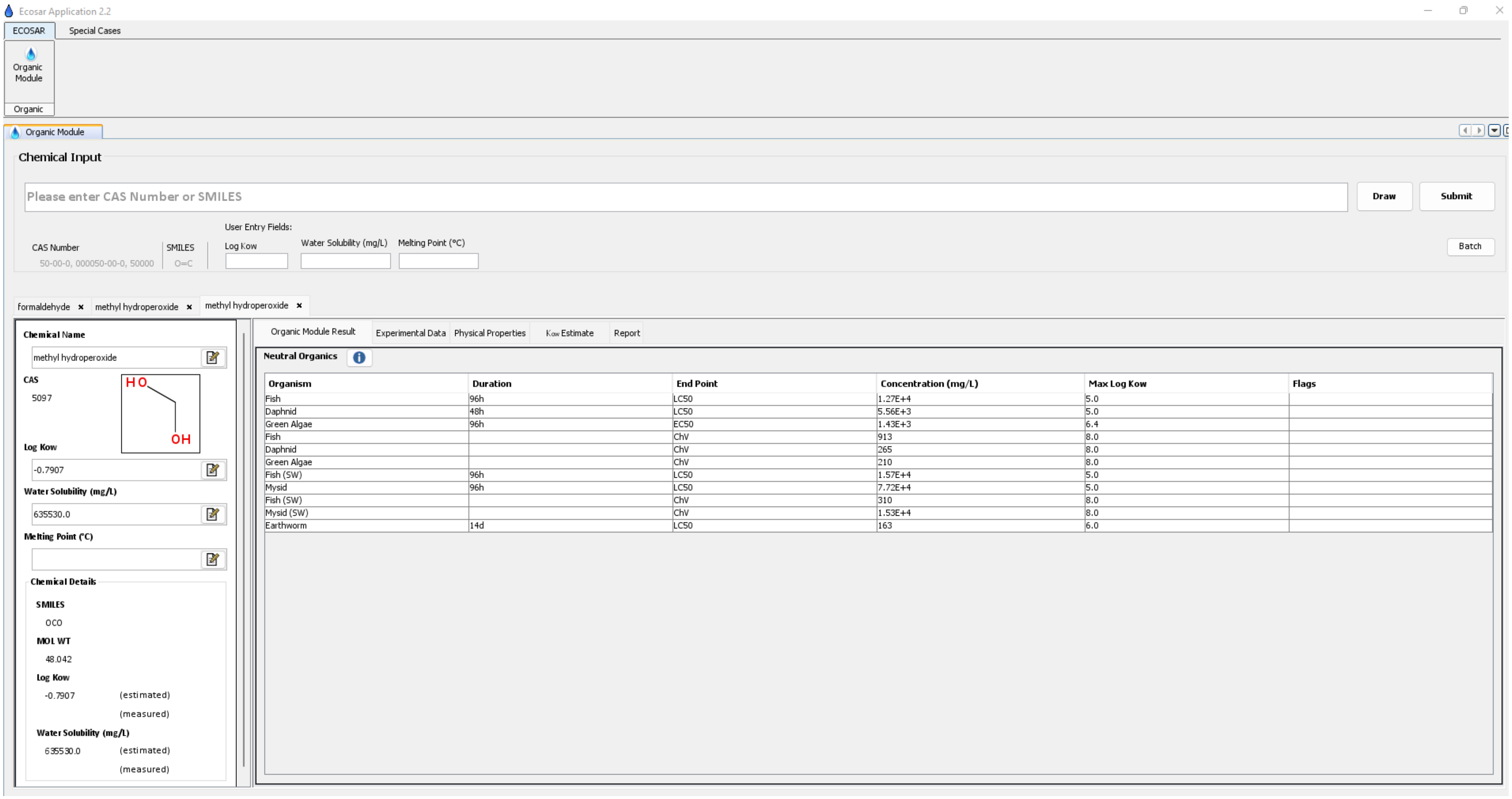



| | Estimate | SE Hessian | LCL Hessian | UCL Hessian | SE Bootstrap | LCL Bootstrap | UCL Bootstrap |
|---|---|---|---|---|---|---|---|
| alpha | 4.5164 | 0.0733 | 4.3728 | 4.6601 | 0.0759 | 4.3717 | 4.6637 |
| beta | 0.1552 | 0.0370 | 0.0827 | 0.2276 | 0.0348 | 0.0847 | 0.2214 |

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

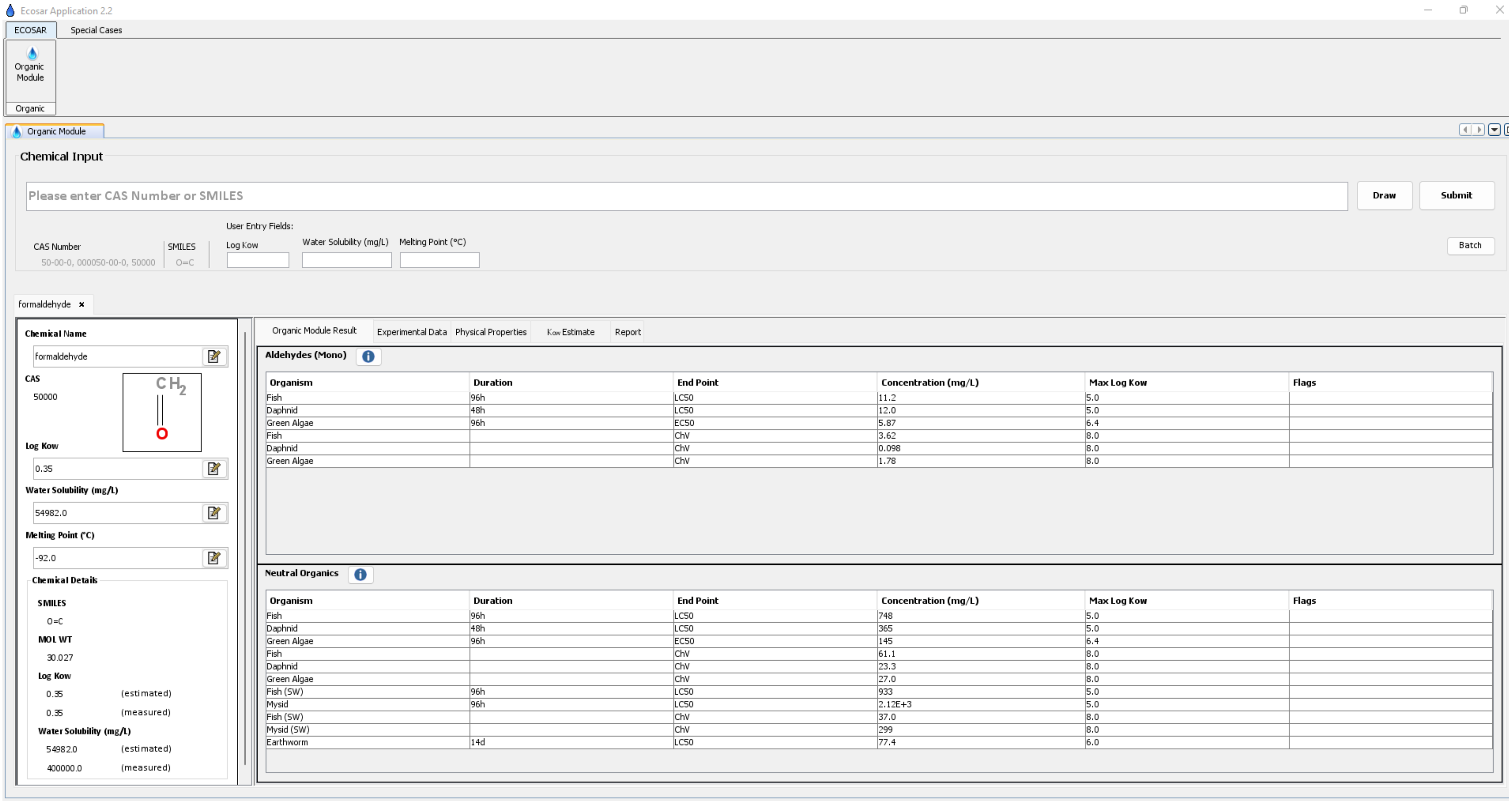
## A.3 Ecological Structure Activity Relationship (ECOSAR) Predictions

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.




| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Fish | 96h | LC50 | 1.27E+4 | 5.0 | |
| Daphnid | 48h | LC50 | 5.56E+3 | 5.0 | |
| Green Algae | 96h | EC50 | 1.43E+3 | 6.4 | |
| Fish | | ChV | 913 | 8.0 | |
| Daphnid | | ChV | 265 | 8.0 | |
| Green Algae | | ChV | 210 | 8.0 | |
| Fish (SW) | 96h | LC50 | 1.57E+4 | 5.0 | |
| Mysid | 96h | LC50 | 7.72E+4 | 5.0 | |
| Fish (SW) | | ChV | 310 | 8.0 | |
| Mysid (SW) | | ChV | 1.53E+4 | 8.0 | |
| Earthworm | 14d | LC50 | 163 | 6.0 | |

**Figure_Apx A-4. ECOSAR Inputs and Outputs for Methylene Glycol (Smiles: 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 the scientific evidence outweighs the uncertainties to the point where it is unlikely that the uncertainties could have a significant effect on the exposure or hazard estimate.
- Moderate (+ +) confidence suggests some understanding of the scientific evidence and uncertainties. The supporting scientific evidence weighed against the uncertainties is reasonably adequate to characterize exposure or hazard estimates.
- Slight (+) confidence is assigned when the weight of the scientific evidence WoSE 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 the 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).