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Office of Chemical Safety and
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Draft Human Health Hazard Assessment for Formaldehyde

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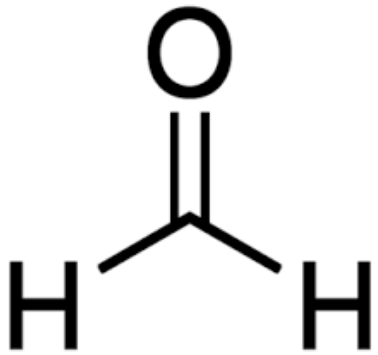

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1 INTRODUCTION

1.1 Background

EPA is currently evaluating risks from formaldehyde under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). This hazard assessment is a collaboration between the Office of Pesticide Programs (OPP) and the Office of Pollution Prevention and Toxics (OPPT), both of which are part of the Office of Chemical Safety and Pollution Prevention (OCSPP). OPP and OPPT intend to use a harmonized suite of human health endpoints and uncertainty/extrapolation factors for evaluating risks from inhalation, dermal, and oral formaldehyde exposure in their respective assessments. This hazard assessment also reflects harmonization with EPA's Office of Research and Development (ORD) and other EPA offices, to the extent appropriate. As a result of this collaboration across programs, multiple federal advisory committees—including the National Academies of Sciences, Engineering, and Medicine (NASEM) and the Human Studies Review Board (HSRB)—have provided review of various aspects of this hazard characterization.

In April 2022, EPA ORD's Integrated Risk Information System (IRIS) published a draft *Toxicological Review of Formaldehyde – Inhalation* (U.S. EPA, 2022) (also called "draft IRIS assessment") of publicly available studies relevant to human health hazards that may result from formaldehyde exposure via inhalation. The draft IRIS assessment was subject to public comment and peer review. In August 2023, NASEM released its *Review of EPA's 2022 Draft Formaldehyde Assessment* (NASEM, 2023). The draft IRIS assessment derived a chronic reference concentration (RfC) for non-cancer risks and an inhalation unit risk (IUR) for cancer risks from inhalation. OPP and OPPT are relying on the draft IRIS assessment for these chronic inhalation hazard values (see Section 1.2.2.1).

OPP and OPPT reviewed available data and identified endpoints and hazard values for dermal, oral and shorter-term inhalation exposure to formaldehyde for use in the FIFRA and TSCA human health risk assessments. This assessment uses data collection and review procedures from both OPP and OPPT such that the relevant hazard assessment materials are the combined results of TSCA systematic review and data quality review processes and OPP's approach to identifying toxicology studies and generation of data evaluation records (DERs). Detailed information on systematic review and data quality evaluation supporting this analysis can be found in the OPP DERs and the OPPT, fit-for-purpose *Systematic Review Protocol for the Draft Risk Evaluation for Formaldehyde* (U.S. EPA, 2023). For dermal and inhalation routes of exposure, formaldehyde has an extensive database of human and animal data. To the extent possible and as appropriate, OPP and OPPT have focused on human studies to avoid animal to human extrapolation. However, for the oral route of exposure, the database of toxicology studies is more limited and is only available from testing in laboratory animals.

EPA consulted with the HSRB on the scientific validity and ethics of four controlled human inhalation studies and a draft weight of evidence (WOE) narrative for a set of acute inhalation points of departure (PODs). The acute inhalation rationale and POD presented in this document have been revised based on the HSRB's feedback. The Agency also consulted with the HSRB on the scientific validity and ethics of two human dermal patch test studies used in this draft assessment to contribute to the WOE and POD derivation for dermal sensitization endpoints.

1.2 Approach to Data Collection and Data Evaluation

This hazard assessment is a collaboration between OPP and OPPT. Each office has a standard process for data gathering, data quality evaluation and data integration, that are typically applied to meet their

respective programmatic needs and statutory obligations. This joint hazard assessment leverages elements of the standard processes of both OPP and OPPT in a fit-for-purpose approach.

1.2.1 Overall Approach

Using the systematic review process, OPPT pre-defines population, exposure, comparator, and outcome (PECO) statements to guide the screening of references. A literature search is conducted using pre-defined search strings, and individual references go through title/abstract and full-text screening to select those relevant for use in chemical-specific risk evaluations. Studies which are determined to be PECO-relevant are evaluated for data quality according to a pre-defined set of criteria outlined and organized according to various domains and metrics. Evaluation criteria used to evaluate animal toxicity studies for formaldehyde were harmonized with the metrics used by IRIS and are available in the *Systematic Review Protocol for the Draft Risk Evaluation for Formaldehyde* (U.S. EPA, 2023). This approach is based on the OPPT systematic review approach described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021) (also called "2021 draft systematic review protocol") but is tailored to the specific needs of the formaldehyde assessment. The output from OPPT's data quality evaluation is a qualitative rating for each metric of critically deficient, low, medium, or high and an overall data quality rating of uninformative, low, medium, or high quality. Since some metrics apply study-wide (*e.g.*, test substance identity) while others are outcome-specific (*e.g.*, outcome assessment methodology), each health outcome (*e.g.*, kidney effects, liver effects) covered by a study can potentially have a different overall data quality rating.

The systematic review protocol provides a framework for considering the usability of individual studies for risk evaluation based on their data quality and provides latitude for studies rated as uninformative to be used in a WOE analysis for hazard identification, but not for dose-response assessment. The process of evidence integration, as depicted in Figure 1-1, comes after data evaluation and extraction. During evidence integration, other studies that may fulfill limitations or address deficiencies may be considered to characterize the hazard of a chemical substance. Data quality evaluation and extraction within each study precedes evidence integration. The integration of separate bodies of evidence (*i.e.*, human, animal, and mechanistic evidence) directly informs the integration across all evidence to draw an overall judgment for each of the assessed human health effects. The evaluation of the strength of the evidence and the weight of the evidence are described more fully in Section 7.5.2 of the 2021 draft systematic review protocol.

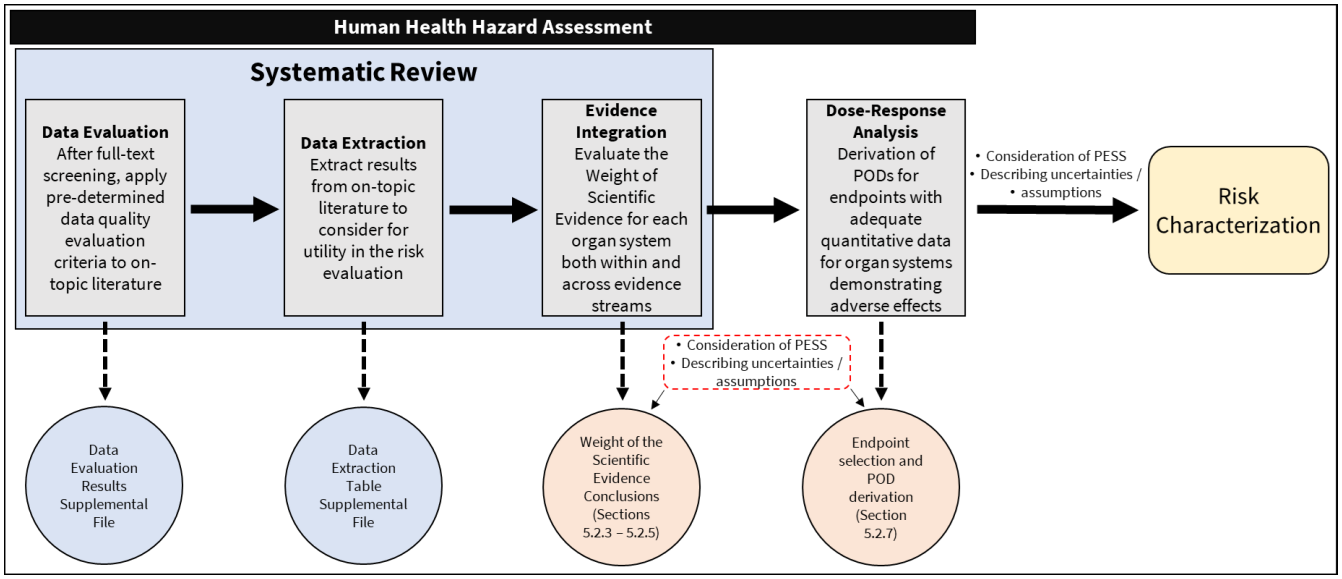

Figure 1-1. OPPT Approach to Hazard Identification, Data Integration, and Dose-Response Analysis

In addition to the studies identified through the OPPT literature search, studies to support data requirements for pesticide registration are also available and considered. Toxicology data requirements for antimicrobial pesticides are identified in 40 CFR Part 158W, which are dependent on the use pattern of the chemical. Studies submitted in response to FIFRA requirements are typically conducted under and evaluated with a series of internationally harmonized and scientifically peer-reviewed study protocols.[1] These guideline 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. For formaldehyde, acute toxicity, dermal sensitization/irritation, mutagenicity/cytogenicity and short-term oral studies were submitted to support pesticide registration, whereas open literature studies were often referenced for chronic toxicity studies. Pesticide regulations provide OPP with the ability to consider non-guideline studies, such as those identified in the open literature or conducted by other federal agencies if they are of sufficient quality. OPP uses its *Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment* to evaluate the quality and utility of open literature studies in a transparent and systematic way. For the current evaluation of formaldehyde, OPP has instead relied upon the OPPT literature search to identify relevant studies for use in the draft formaldehyde risk evaluation.

In addition to the data quality evaluation for individual studies performed as part of the OPPT systematic review process, OPP developed DERs to independently evaluate study quality of all key studies used in support of dose-response analysis. Study DERs are publicly accessible documents that are generated in accordance with standardized, harmonized templates[2] that ensure consistent information and review. DERs include a summary of the study methods, observations, and results, as well as OPP reviewer interpretation and conclusions. Detailed reporting tables are also included for all effects where there were significant differences from the control. Draft DERs are reviewed by at least two scientists for accuracy and consistency with OPP guidance on interpretation of toxicity studies.

[1] https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines.
[2] https://www.epa.gov/pesticide-registration/study-profile-templates#toxicology.

In cases where human studies were identified, consistent with EPA's obligations under its Human Studies Rule, specifically 40 CFR subpart P, OPP and OPPT reviewed these studies to ensure they were scientifically valid and ethically conducted. EPA then consulted with the HSRB on these study reviews. The HSRB is a federal advisory committee that operates in accordance with the provisions of the Federal Advisory Committee Act (FACA), 5 U.S.C. § 10. The HSRB is required to review and comment on all proposed and completed third-party research (*i.e.*, research that is not conducted or sponsored by the Federal government) involving intentional human subject exposure that is subject to the coverage of EPA's regulations (see subparts K–L). The HSRB provides advice and recommendations on scientific and ethical considerations of these studies to the EPA through a written report.

1.2.2 Route-Specific Considerations and Results

The overall approach described above was utilized to screen data to determine what information is relevant and impactful for risk assessment. The way these processes are integrated is specific to each route of exposure as further discussed below.

1.2.2.1 Inhalation

For the inhalation route, OPP and OPPT relied on the systematic review performed to support the draft draft IRIS assessment. Using the OPPT literature search process described above, an additional search was performed to identify any studies that may not have been captured by the IRIS search. Twelve additional inhalation studies were identified that were not included in the draft IRIS assessment. However, the critical cancer and non-cancer *health outcomes* described in these studies are already captured in the draft IRIS assessment. Of the 12 studies, 5 (Rea and Pan, 2000; Eberlein-König et al., 1998; Górski et al., 1992; Reed and Frigas, 1985; Weber et al., 1976) did not provide sufficient dose-response information and therefore were not further considered. While seven studies (Garrett et al., 1997; Menzies et al., 1996; Milton et al., 1996; University of Pittsburgh, 1992; Godish, 1990; Lamm, 1984; U.S. EPA, 1983) did provide dose-response information, none described effects that were more sensitive than the studies in the draft IRIS assessment. Therefore, these studies were not further assessed for use in the OPP or OPPT assessment. OPP and OPPT are relying on the chronic cancer and non-cancer hazard values derived in the draft IRIS assessment.

Although the draft IRIS assessment was designed to derive hazard values for chronic inhalation exposure, it included identification of acute non-cancer endpoints, as well as data quality evaluation and dose-response analysis for key studies. The underlying systematic review process and dose-response analysis performed for acute endpoints in the draft IRIS assessment thus provided a foundation for OPP and OPPT's evaluation of acute inhalation endpoints. To complement the analysis completed by IRIS, the overall systematic review approach described above was used to identify additional relevant human evidence to consider for acute inhalation hazards. Because of the extent of human data available for formaldehyde, EPA did not formally review studies in animals. Integrating data quality review methods used in both OPP and OPPT, key studies were identified relevant to endpoint selection and POD identification. DERs were prepared for these studies critical to POD determination using OPP DER templates and processes. Four human studies considered useful for WOE were evaluated according to the standards in the Human Studies Rule at 40 CFR 26 for scientific and ethical conduct (CFR, 2024). EPA's reliance on the studies complies with the relevant standards in that regulation.

1.2.2.2 Dermal

For dermal hazard characterization, the systematic review identified both human and animal studies reporting effects of formaldehyde through dermal exposure. Two animal studies were identified to inform the cancer potential following dermal exposure to formaldehyde. OPP and OPPT focused its non-cancer review on those studies that evaluated the most sensitive endpoints at lower dose levels.

Integrating data quality review methods used in both OPP and OPPT, key studies relevant to endpoint and POD selection were identified, which included one animal study and two human studies. DERs were prepared for these using OPP DER templates and processes. The two human studies were evaluated according to the standards in the Human Studies Rule at 40 CFR 26 for scientific and ethical conduct (CFR, 2024). EPA's reliance on the studies complies with the relevant standards in that regulation.

Additionally, OPP and OPPT also considered *in vitro* data based on OPP's previous work using quantitative risk assessment for skin sensitization (U.S. EPA, 2020) where these data were used to establish quantitative endpoints for induction thresholds for skin sensitization. OPP worked with the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to generate predicted EC3 values for the isothiazolinone chemistries based on the Model 4 artificial neural network (ANN) models described in Hirota et al., (2015). Similar data and model results are available for formaldehyde that were incorporated into the dermal WOE.

1.2.2.3 Oral

For oral exposure hazard characterization, EPA did not identify any human studies that provide direct quantitative information about the effects of oral exposure to formaldehyde. The systematic review identified animal studies that evaluated non-cancer and cancer effects of formaldehyde through oral exposure. Five animal studies were identified that evaluated the carcinogenic potential of formaldehyde following oral exposure. Based on integrated data quality review methods from both offices, OPP and OPPT identified key studies relevant to endpoint selection and POD identification. DERs were prepared for three studies critical to POD determination using OPP's DER templates and processes. Key studies utilized for oral POD determination also underwent additional intra-agency review by OPP's Health Effects Division, OPPT's New Chemical Division, and ORD's Chemical and Pollutant Assessment Division.

2 ABSORPTION, DISTRIBUTION, METABOLISM, ELIMINATION (ADME)

This section summarizes the absorption, distribution, metabolism, and elimination (ADME) data available for formaldehyde based primarily on information reported in a more comprehensive discussion of toxicokinetics in the draft IRIS assessment (U.S. EPA, 2022). Information on the dermal and oral pathways is based on review of relevant studies by OPP and OPPT.

Formaldehyde is a small aldehyde (30 g/mol) and a gas at room temperature. It is water soluble and reactive and will, therefore, react chemically at the site of first contact in biological systems. It is readily absorbed by all routes and reacts with both high and low molecular weight compounds. Formaldehyde in biological systems is well understood to exist as a dynamic equilibrium between the hydrated and unhydrated forms. In water, the majority of formaldehyde exists as the hydrated form, methylene glycol (CH2(OH)2) and less than 0.1 percent exists unhydrated (Priha et al., 1996). Because the hydration reaction favors methylene glycol, exogenous formaldehyde in the blood will exist primarily as methylene glycol and thus be physiologically eliminated (exhalation, urine, feces). The free unhydrated formaldehyde will react with serum proteins and cellular components.

2.1 Inhalation

As described in the draft IRIS assessment (U.S. EPA, 2022), formaldehyde is readily absorbed by respiratory tract tissues and both human and animal dosimetric modeling studies indicate that 90 to 95 percent of inhaled formaldehyde is deposited in the upper respiratory tract (URT). Most studies indicate that formaldehyde does not usually distribute into the lower respiratory tract, unless the individual is exposed repetitively or if their ventilation rate changes, as with occupational exposures. Certain formaldehyde-related effects might affect the distribution of formaldehyde. Damage to the mucociliary apparatus, the respiratory tract's first line of defense, may result in increased distribution to the lower respiratory tract and subsequently increased systemic absorption of formaldehyde.

As further described in the draft IRIS assessment, once in the URT, formaldehyde is primarily metabolized by glutathione-dependent class III alcohol dehydrogenase (ADH3) and aldehyde dehydrogenase 2 (ALDH2) to formate. Additionally, formaldehyde has been shown to non-covalently bind to multiple compounds, such as glutathione (GSH), tetrahydrofolate (THF), and albumin in nasal mucus. Formaldehyde can also covalently bond to macromolecules forming DNA-protein crosslinks (DPXs), DNA-DNA crosslinks (DDCs), hydroxymethyl-DNA (hm-DNA) adducts, or protein adducts, such as N6-formyllysine as evidenced in rat and monkey studies.

The draft IRIS report also includes a robust discussion of the potential for systemic delivery of inhaled formaldehyde to distant sites. IRIS cited several studies supporting that exogenous formaldehyde is neither systemically distributed nor significantly absorbed into blood. As summarized by NASEM in their review of the draft IRIS assessment, "EPA concluded that inhaled formaldehyde is not distributed to an appreciable extent beyond the respiratory tract to systemic sites; thus, inhaled formaldehyde is not directly interacting with tissues distal to the portal of entry to elicit effects" (NASEM, 2023) (p. 46). Detailed discussions are available in the draft IRIS and NASEM reports.

2.2 Dermal

Several studies evaluate dermal absorption of formaldehyde. In an *in vitro* flow-through diffusion cell (Lodén, 1986), formaldehyde absorption was reported at 319 µg/cm^2/hour for a 37 percent formalin solution, and 16.7 µg/cm^2/hour for a 10 percent phosphate buffered formaldehyde solution. Two studies in rats report absorption of roughly 6 to 9 percent of applied formaldehyde following dermal doses

ranging from 0.1 to 2 mg formaldehyde (Bartnik et al., 1985; Jeffcoat et al., 1983). These studies indicate that dermal absorption of formaldehyde can occur (<10%); however, dermal absorption factors are not needed for this hazard assessment as endpoints are based on skin sensitization observed in human dermal studies.

2.3 Oral

Formaldehyde is absorbed from the gastrointestinal tract following ingestion. Oral absorption of [^{14}C]-formaldehyde (7 mg/kg) in rats resulted in 40 percent elimination as exhaled ^{14}C-carbon dioxide ($^{14}CO_2$), with 10 percent excretion in urine, 1 percent excretion in feces, and much of the remaining 49 percent retained within the carcass—presumably due to metabolic incorporation (IARC, 1995; Buss et al., 1964). An oral study looked at the complexes between ^{14}C-formaldehyde and milk proteins with male Sprague Dawley rats and CD-1 mice. The study, in which rats and mice were fed a single dose (2.2 g/18 µCi for rats and 0.5 g/4 µCi for mice) of grana cheese made from milk with added [^{14}C]-formaldehyde, revealed that within 32 hours of ^{14}C-formaldehyde ingestion 67 and 64 percent of the radioactivity, respectively, had been excreted in feces and urine, 28 and 24 percent, respectively, were exhaled, indicating absorption of the ingested dose (Galli et al., 1983).

3 CANCER HAZARD CHARACTERIZATION

In accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), and as described in more detail in the draft IRIS assessment (U.S. EPA, 2022), EPA characterized the available evidence for a range of upper respiratory tract cancers and non-respiratory cancers resulting from inhalation exposure to formaldehyde. This section summarizes key conclusions of the draft IRIS assessment on cancer risks from inhalation exposures and describes available evidence identified by OPP and OPPT through systematic review of oral and dermal exposure studies in animals.

3.1 Inhalation

OPP and OPPT rely on the cancer conclusions for formaldehyde inhalation presented in the draft IRIS assessment and peer reviewed by NASEM. Based on available human and animal data, the draft IRIS assessment evaluated the WOE and performed dose-response analysis for several respiratory and non-respiratory cancer types to derive an inhalation unit risk (IUR).

IRIS concluded that formaldehyde is carcinogenic to humans by the inhalation route of exposure based on several lines of evidence. Specifically, IRIS concluded that "evidence demonstrates that formaldehyde inhalation causes nasopharyngeal cancer, sinonasal cancer and myeloid leukemia in exposed humans, given appropriate exposure circumstances." IRIS also evaluated available evidence for other respiratory and non-respiratory cancer types, although these did not contribute to the overall cancer hazard conclusions.

3.1.1 Inhalation Unit Risk

OPP and OPPT rely on the IUR derived in the draft IRIS assessment on formaldehyde and peer reviewed by NASEM. Based on available human and animal data, the draft IRIS assessment evaluated the WOE and performed dose-response analysis for a range of cancer effects to derive an IUR.

In the draft IRIS assessment, IRIS derived IUR estimates based on nasopharyngeal cancer in humans and squamous cell carcinoma in the respiratory tract in animals (U.S. EPA, 2022). IRIS also explored derivation of the IUR based on myeloid leukemia in humans. Although there is strong evidence that formaldehyde exposure causes myeloid leukemia in humans, uncertainties in the available dose-response data reduced IRIS's confidence in the quantitative IUR estimate derived for myeloid leukemia. IRIS therefore identified the IUR derived based on nasopharyngeal cancer in humans (Table 3-1) as the preferred IUR for quantitatively evaluating cancer risk from inhaled formaldehyde.

Table 3-1. Inhalation Unit Risk for Formaldehyde as Presented in the Draft IRIS Assessment

Cancer Type	Lifestage Adjustment	Preferred Unit Risk Estimate (Ppm^{-1})	Preferred Unit Risk Estimate ($[mg/m^3]^{-1}$)
Nasopharyngeal	Adult-based[a]	0.0079	6.4E–06
	ADAF-adjusted[b]	0.013	1.1E–05

[a] adult-based unit risk estimate for application in exposure scenarios with no early life exposure or for scenario-specific age-dependent adjustment factor (ADAF) adjustment
[b] ADAF-adjusted IUR for application in lifetime exposure scenarios

3.1.2 Age-Dependent Adjustment Factor

Based on the mode of action analysis presented in Sections 1.2.5 and 1.3.3 of the draft IRIS assessment, IRIS concluded there is sufficient evidence that a mutagenic mode of action contributes to risk of nasopharyngeal cancer from inhaled formaldehyde. Similarly, NASEM review concluded that "While there is uncertainty in the degree to which nonmutagenic processes may also contribute to the carcinogenic activity of formaldehyde inhalation at the point-of-entry tissues, there is sufficient evidence to support the assumption that a mutagenic MOA is involved in the carcinogenesis of formaldehyde in the upper aerodigestive tract in humans" (NASEM, 2023). When a mutagenic mode of action contributes to cancer risk, EPA cancer guidelines (U.S. EPA, 2005b) recommend that cancer risk estimates incorporate age-dependent adjustment factors (ADAFs) to account for the potential for greater susceptibility associated with early life exposure.

3.2 Dermal

OPP and OPPT did not identify any human studies quantitatively evaluating the relationship between dermal exposure to formaldehyde and cancer in humans. Two studies in mice evaluated tumor formation and tumor promotion following dermal exposure to formaldehyde (Iversen, 1988; Company Withheld, 1984), but both have limitations (U.S. EPA, 2023) that reduce confidence in the results.

OPP and OPPT have not made a determination regarding the carcinogenic potential of formaldehyde through dermal exposure. However, there is no direct evidence of the carcinogenicity of formaldehyde following dermal exposure.

3.3 Oral

OPP and OPPT did not identify any studies evaluating the relationship between oral exposure to formaldehyde and cancer in humans.

Five animal studies (Soffritti et al., 2016; Soffritti et al., 2002; Soffritti et al., 1989; Til et al., 1989; Tobe et al., 1989; Civo Institute TNO, 1987a; Takahashi et al., 1986) have evaluated the carcinogenicity of oral exposure to formaldehyde. Three of the five studies report increased tumor incidence following oral exposure to formaldehyde. However, all of the studies have major limitations (U.S. EPA, 2023) that make it difficult to interpret the results with confidence.

OPP and OPPT have not made a determination regarding the carcinogenic potential of formaldehyde through oral exposure. However, there is little direct evidence of the carcinogenicity of formaldehyde following oral exposure.

4 NON-CANCER HAZARD CHARACTERIZATION

This section summarizes the range of human health hazard effects associated with formaldehyde. Evidence presented below for effects associated with inhalation exposures is primarily summarized from the draft IRIS assessment for formaldehyde but also includes information gathered through systematic review. Evidence presented below for effects associated with oral and dermal exposure routes is drawn from results of the OPP and OPPT data gathering processes.

4.1 Inhalation

4.1.1 Summary of Hazard Endpoints

For inhalation hazard characterization, OPP and OPPT rely on the draft IRIS assessment. This section provides a brief summary of the effects of inhalation exposure to formaldehyde described in the draft IRIS assessment, which is primarily focused on chronic exposures; however, the draft IRIS assessment also included identification of acute endpoints and dose-response analyses for key studies that inform the OPP and OPPT evaluations.

Sensory Irritation

Formaldehyde is a sensory irritant of the eyes and respiratory tract, with symptoms ranging from mild to severe including itching, burning, stinging sensations, watering eyes, sneezing, rhinitis, sore throat, coughing and bronchial constriction. IRIS concluded that the evidence demonstrates that inhalation of formaldehyde causes sensory irritation in humans, given appropriate exposure circumstances.

Sensory irritation in response to formaldehyde has been reported in multiple controlled human exposure studies (Mueller et al., 2013; Lang et al., 2008; Kulle et al., 1987; Andersen and Molhave, 1983) as well as observational epidemiology studies (Liu et al., 1991; Hanrahan et al., 1984). In controlled human exposure experiments, these symptoms have been shown to occur within seconds at high enough doses (Andersen and Molhave, 1983). Sensory irritation in humans has been reported at concentrations as low as 0.08 ppm (0.1 mg/m^3) and resolve when exposure is stopped (Andersen and Molhave, 1983; Andersen, 1979).

As noted in the draft IRIS assessment (U.S. EPA, 2022), sensory irritation is "understood to occur as a result of direct interactions of inhaled formaldehyde with cellular macromolecules in the nasal mucosa leading directly or indirectly to stimulation of trigeminal nerve endings located in the respiratory epithelium" (pp. 1–11).

Pulmonary Function

IRIS concluded that evidence indicates that long-term inhalation of formaldehyde likely causes decreased pulmonary function in humans given the appropriate exposure circumstances.

Immune-Mediated Effects: Allergies and Asthma

IRIS concluded that evidence indicates that inhalation of formaldehyde likely causes an increased risk of prevalent allergic conditions and prevalent asthma symptoms, as well as decreased control of asthma symptoms, given appropriate exposure circumstances (U.S. EPA, 2022).

Respiratory Tract Pathology
IRIS concluded that the evidence demonstrates that inhalation of formaldehyde causes respiratory tract pathology (primarily squamous metaplasia) given the appropriate exposure circumstances (U.S. EPA, 2022).

Reproductive and Developmental Effects
IRIS concluded that the evidence indicates that inhalation of formaldehyde likely causes increased risk of developmental, and female and male reproductive toxicity given the appropriate exposure circumstances (U.S. EPA, 2022).

Neurological Effects
IRIS concluded that the evidence suggests but is not sufficient to infer that formaldehyde inhalation might cause multiple manifestations of nervous system health effects in humans given relevant exposure circumstances (U.S. EPA, 2022).

4.1.2 Identification of Endpoints for Dose-Response Analysis and POD Derivation

4.1.2.1 Acute

OPP and OPPT selected sensory irritation as the basis for acute POD derivation. Although the draft IRIS assessment was focused on chronic inhalation, it also identified sensory irritation as an endpoint relevant for acute exposures because the effect occurs as an immediate response to an exposure. For other endpoints evaluated in the draft IRIS assessment (pulmonary function, immune-mediated conditions, asthma prevalence and control, respiratory tract pathology, nervous system effects, developmental toxicity, and male and female reproductive toxicity), IRIS concluded that the available studies for those health outcomes either do not provide clear evidence of acute effects or do not provide sufficient information to support dose-response analysis for acute exposures. For immune-mediated conditions, including asthma, acute exposures may be of concern, but the available studies do not provide sufficient information to support dose-response analysis for acute exposures.

Use of sensory irritation is consistent with other national and international exposure limits derived under a range of regulatory and advisory contexts for general population and occupational exposures which have consistently been based on sensory irritation endpoints (Appendix A). Because of the extent of human data available for formaldehyde, EPA did not formally review any evidence of sensory irritation in animals. However, the draft IRIS assessment did summarize the available mechanistic evidence for sensory irritation in animals (U.S. EPA, 2022).

The sensory irritation effects of formaldehyde appear to be more responsive to the exposure concentration than to exposure duration and may not adhere to Haber's law (Shusterman et al., 2006). Based on review of the weight of evidence analysis presented to the HSRB in May 2023, the HSRB did not recommend duration adjustments for 8- or 24-hour PODs for the sensory endpoint, based on the lack of support for this adjustment in the four studies presented in the WoE and the existing literature (HSRB, 2023a). Therefore, this analysis focuses on identifying peak threshold concentration levels that may result in sensory irritation, rather than deriving duration-adjusted acute PODs for 8- and 24-hour average concentrations.

OPP and OPPT identified four controlled human exposure studies (Mueller et al., 2013; Lang et al., 2008; Kulle et al., 1987; Andersen and Molhave, 1983) to inform selection of an acute peak exposure level (summarized in Table 4-1). HSRB agreed with EPA's conclusions that each of the studies discussed below were scientifically sound and ethically conducted that could be used quantitatively or

qualitatively to support the acute inhalation WOE. The feedback from HSRB was incorporated into the final DERs prepared for each specific study and is reflected in the discussion below. All of the studies were classified in the DERs as acceptable/non-guideline.

Kulle et al., (1993; 1987) is a controlled human exposure study conducted in healthy male and female volunteers (n = 10–19). Volunteers were exposed to formaldehyde (0.5 to 3 ppm) for 3 hours on 5 occasions, with exercise during some exposure periods. Sensory irritation was self-reported before, during, and after exposures. There was increased incidence of reported odor and eye irritation with concentration. After exposure to 0.5 ppm for 3 hours, no subjects reported eye irritation. At the 1.0 ppm formaldehyde exposure concentration, 4 of 19 subjects[3] reported mild eye irritation and 1 reported moderate eye irritation. At the 2.0 ppm exposure concentration, 6 subjects reported mild irritation and 4 reported moderate eye irritation. Linear trends for increased odor and eye irritation ($p < 0.0001$) were observed from statistical analysis in Group II subjects exposed at rest. Nasal resistance was significantly increased at the 3.0 ppm formaldehyde concentration and was increased but not significant at 2.0 ppm.

When analyzing pulmonary function, Kulle et al., (1993; 1987) found no significant decrements or increases in bronchial reactivity to methacholine (a standard substance used to assess bronchial airway reactivity) observed at any formaldehyde concentration tested, at rest or after exercise. Exercise during this study was observed to increase the incidence of nose/throat irritation but not the eye irritation or odor threshold response. Following review, IRIS rated this study with an overall confidence level of medium. The HSRB agreed with the EPA's assessment of this study as scientifically sound and ethically conducted and provides reliable data to use in a WOE (HSRB, 2022).

Andersen et al., (1983; 1979) is a controlled human exposure study in healthy and smoker male and female volunteers (n = 16). Sensory irritation was self-reported by subjects indicating degree of irritation on a 1 to 100 scale during exposure, and eye blinking was measured. There were four controlled conditions: 0.24, 0.40, 0.81, and 1.61 ppm formaldehyde, lasting for 5 hours each. These concentrations were administered on four different days with each subject serving as their control. Nasal mucociliary flow was observed in the anterior portion of the nasal turbinates and was found to be significantly decreased at the 0.24 ppm concentration. There was no further reduction in flow rate at 0.40 ppm and above. In contrast, the posterior portion of the nasal turbinates was not affected. In the middle third of the nasal turbinates, there was no significant difference on reduction of average mucociliary flow rate between 1 to 3 hours and 4 to 5 hours exposure.

Airway resistance measurement results in Andersen et al., (1983; 1979) showed no significant effect of formaldehyde inhalation exposure on vital capacity, forced expiratory flow, or forced expiratory volume at any concentration tested. Similarly, irritation assessment results indicated that after 2 hours exposure, there was no reported discomfort after exposure to 0.24 or 0.40 ppm. In the remaining part of the exposure period (presumably 4 to 5 hours), discomfort was reported at 0.24 and 0.40 ppm. At 0.81 and 1.6 ppm, discomfort was reported in the first hour of exposure. Subjectively, test subjects reported conjunctival irritation and dryness of the nose and throat following formaldehyde exposures. The incidence of reported symptoms was 3, 5, 15, and 15 subjects in the 0.24, 0.40, 0.81, and 1.6 ppm exposure groups respectively. These symptoms had dissipated by the following morning. IRIS rated this study with an overall confidence rating of medium. The HSRB agreed with the EPA's assessment of this study as scientifically sound and ethically conducted, and recommended, with caveats, that Andersen and Mølhave (1983), a book chapter that reports results from the 1979 study, could be used qualitatively to support a WOE (HSRB, 2022).

[3] Values based on 1993 reanalysis.

Lang et al., (2008) is a controlled human exposure study in healthy non-smoking adult volunteers (n = 21). There were ten controlled exposure conditions that were administered for 4 hours each over 10 days: clean air, 0.15, 0.3, and 0.5 ppm; additional 0.3 and 0.5 ppm with peaks up to 1.0 ppm. Sensory irritation was assessed by blinking frequency, conjunctival redness, nasal flow, and resistance, and via a questionnaire. There were no significant effects of treatment on nasal flow and resistance, pulmonary function, and reaction times. Blinking frequency and conjunctival redness significantly increased at 0.5 ppm with short-term peak exposures of 1.0 ppm (0.5/1.0 ppm). Subjective ratings reported eye and olfactory symptoms as low as 0.3 ppm. Nasal irritation symptoms were reported at 0.5/1.0 ppm and at 0.3 ppm and 0.5 ppm with co-exposure to ethyl acetate (EA) ($p < 0.05$). EA alone was also reported as irritating.

When Lang et al., (2008) considered personality traits, volunteers who rated as anxious tended to report complaints at a higher intensity and when "negative affectivity" was used as a covariate, 0.3 ppm dropped out as an effect level, but 0.5/1.0 ppm remained statistically significant for eye and nasal irritation and olfactory symptoms. IRIS rated this study with an overall confidence rating of high. The HSRB agreed with the EPA's assessment of this study as scientifically sound and ethically valid, providing reliable data for use in a WOE (HSRB, 2023a).

Mueller et al., (2013) is a controlled human exposure study in hypersensitive and hyposensitive healthy non-smoking adult male volunteers (n = 41). There were five controlled exposure conditions administered for 4 hours each over 5 days, with 15-minute peaks in exposure (clean air, 0.3 + 4 peaks of 0.6 ppm, 0.4 + 4 peaks of 0.8 ppm, 0.5 ppm, and 0.7 ppm). Sensory irritation was assessed by blinking frequency and conjunctival redness, tear film break-up time, nasal flow, and resistance, and via a questionnaire. Results indicated that there were no exposure-related effects on conjunctival redness and blinking frequency. Tear film break-up time increased in the 0.4/0.8 ppm and 0.5 ppm exposure groups ($p < 0.05$) (both hypo- and hypersensitive individuals). Nasal flow rates increased in hypersensitive subjects at 0.7 ppm ($p < 0.01$).

In Mueller et al., (2013), the Swedish Performance Evaluation System (SPES) (Seeber et al., 2002; Gamberale, 1989) subjective survey sum score showed a statistically significant increase in hypersensitive subjects at 0.3/0.6 ppm ($p < 0.001$) and 0.4/0.8 ppm ($p < 0.01$); the perception of impure air increased in hypersensitive subjects at all exposure levels (including clean air, 0.01 ppm). Combined eye symptom survey scores were reported to be higher among hypersensitive subjects at all exposure concentrations except 0.7 ppm (0.86 mg/m^3). Changes in scores were not statistically significant, and no exposure-response was observed. When controlled for "negative affectivity" these associations were not altered (indicating negative personality traits did not affect symptom reporting). IRIS rated this study with an overall confidence rating of high. The HSRB also agreed with the EPA's assessment of this study as scientifically sound, providing reliable data for use in a WOE (HSRB, 2023a).

Table 4-1. Key Human Studies Used to Evaluate Peak Air Concentrations of Formaldehyde Associated with Sensory Irritation

Source	Exposure Concentrations	Effects
Kulle (1993); Kulle et al. (1987)	I: 0.0, 0.5, 1.0, 2.0 ppm, 2.0 ppm exercise II: 0.0, 1.0, 2.0 ppm, 2.0 ppm exercise I: 0, 0.62, 1.23, 2.46, mg/m^3 II: 0, 1.23 3.69 mg/m^3	NOAEL =0.5 ppm (0.62 mg/m^3) LOAEL = 1.0 ppm (1.23 mg/m^3) for mild to moderate eye irritation BMC = 0.69 ppm (0.85 mg/m^3) BMCL =0.502 ppm (0.617 mg/m^3)
1983); Andersen (1979)	0.24, 0.4, 0.81, 1.61 ppm 0.3, 0.5, 1.0, 2.0 mg/m^3	During first 2 hours, no reported irritation discomfort to 0.24 or 0.4 ppm but discomfort to 0.81 and 1.61 ppm within the first hour. During remaining 3 hours exposure, discomfort reported at the 0.24 and 0.4 ppm exposure levels.
Lang et al. (2008)	0, 0.15, 0.3, 0.5 ppm 0.3/0.6, 0.5/1.0 ppm peaks (0, 0.3, 0.5 ppm with EA) 0, 0.19, 0.37, 0.62 mg/m^3 0.37/0.74, 0.62/1.23 mg/m^3 peaks (0, 0.37, 0.62 mg/m^3 with EA)	NOAEL = 0.5 ppm continuous (0.62 mg/m^3) and 0.3 ppm with peak 0.6 ppm (0.37/0.74 mg/m^3) LOAEL = 0.5 ppm with peaks of 1 ppm (0.62/1.23 mg/m^3) for blinking frequency, conjunctival redness, eye and nasal irritation, and olfactory symptoms
Mueller et al. (2013)	0, 0.5, 0.7 ppm 0.3/0.6 ppm peaks, 0.4/0.8 ppm peaks 0, 0.62, 0.86 mg/m^3 0.37/0.74 mg/m^3 0.49/0.98 mg/m^3	At 0.3/0.6 ppm, increase in reported irritation in hypersensitive individuals. 0.4/0.8 ppm increase in reported irritation in hypersensitive individuals and tear film break-up time. 0.7 ppm statistically significant increase in nasal flow in hypersensitive males. For hyposensitive males: 0.4/0.8 ppm and 0.5 ppm increase in tear film break-up time
NOAEL = no-observed-adverse-effect-level; LOAEL = lowest-observed-adverse-effect-level; BMC= benchmark concentration; BMCL = benchmark concentration level (lower 95% confidence limit).		

Additional human evidence for sensory irritation was summarized in the draft IRIS assessment. Two observational epidemiology studies reported associations between residential formaldehyde exposures and self-reported sensory irritation effects (Liu et al., 1991; Hanrahan et al., 1984). While these observational epidemiology studies provide additional information on sensory irritation effects, they measure effects over a much longer duration than the controlled exposure studies and are less directly informative for derivation of an acute peak exposure level. These studies are therefore not considered for dose-response analysis for acute POD derivation.

For each of the four key studies, OPP and OPPT considered dose-response information to identify concentrations associated with sensory irritation over relatively short exposure durations. To identify peak air concentrations associated with immediate sensory irritation responses, OPP and OPPT focused on studies that evaluated shorter duration exposures. Two of the studies directly evaluated effects of 15-minute peaks in exposure during 4-hour exposure periods, while the others evaluate effects following 2 to 5 hours of exposure at a consistent level.

POD Derivation

PODs were derived for each of the three studies that the HSRB supported using quantitatively. An acute threshold POD was selected based on the 0.5 ppm no-observed-adverse-effect-concentration (NOAEC) (and corresponding BMCL, also 0.5 ppm) identified for a 3-hour exposure in Kulle et al., (1993; 1987).

Table 4-2. Candidate Acute Inhalation PODs Based on Sensory Irritation

Citation	Exposure Scenario	Candidate POD	Relevant Ufs	Total UF
Kulle (1993); Kulle et al. (1987)	Continuous 3-hour exposures, with exercise during some exposure periods (healthy adult volunteers)	NOAEC = 0.5 ppm (0.62 mg/m^3) for continuous exposure	UF_H = 10	10
Lang et al. (2008)	Continuous 4-hour exposures to clean air, 0.15, 0.3, and 0.5 ppm 4-hour exposure to 0.3 and 0.5 ppm with 15-minute peaks up to 1.0 ppm (healthy adult volunteers)	NOAEC = 0.5 ppm (0.62 mg/m^3) for continuous exposure NOAEC= 0.3 ppm for 4 hours with 0.6 ppm 15 min peak (0.37/0.74 mg/m^3) exposure	UF_H = 10	10
Mueller et al. (2013)	4-hour exposures to 0.3, 0.4 or 0.5 ppm with 15-minute peaks in exposure 0.6, 0.8 or 0.7 ppm (hypersensitive or hyposensitive healthy non-smoking adult male volunteers)	LOAEC = 0.3 ppm for 4 hours with 0.6 ppm peak exposure (0.37/0.74 mg/m^3) in hypersensitive individuals	UF_H = 10	10

Acute POD (threshold) = 0.5 ppm (0.62 mg/m^3)
UF = 10× (UF_H = 10)

The selected POD is supported by the other three co-critical studies. The POD of 0.5 ppm is

- equal to NOAEL identified for sensory irritation over a 4-hour exposure in Lang et al., (2008);
- below the 0.6 ppm 15-minute peak exposure concentration identified as a LOAEL in hypersensitive individuals in Mueller et al., (2013);
- below the 0.6 ppm 15-minute peak exposure concentration identified as the NOAEL in Lang et al., (2008); and
- consistent with the LOAEL of 0.8 ppm and corresponding NOAEL of 0.4 ppm following 2 hours of exposure reported in Anderson and Mølhave, (1983).

Application of an UF for interindividual variability is consistent with irritation reported by Mueller in hypersensitive individuals following exposure to 0.3 ppm with peak exposures of 0.6 ppm. It is also consistent with high variability across individuals reported in all controlled exposure studies.

Sources of Confidence and Uncertainties

The acute POD is based on a robust dataset, including four high-quality controlled exposure studies with relevance for acute exposure scenarios. OPP and OPPT identified sensory irritation as the most sensitive endpoint for which acute dose-response data are available. Concordance of reported sensory irritation effects and the effect levels reported across all four of these acute exposure studies increases confidence in the final POD.

Variability across individuals' response contributes to uncertainty around effect levels that are protective across the population. Observational epidemiology evidence in Liu et al., (1991) suggests that some individuals (*e.g.*, those with chronic respiratory conditions) may be more susceptible to sensory irritation. Application of a 10× uncertainty factor is applied to account for uncertainty related to intraindividual variability.

This acute POD focuses on defining peak threshold exposure concentrations rather than average 8- or 24-hour exposure concentrations. There is some uncertainty around the degree to which duration influences effect levels because there are no studies available that provide direct evidence that effect levels following 8- or 24-hour exposures are the same as effects following 2 to 5 hours of exposure; As described above, effects cannot be extrapolated because formaldehyde does not follow Haber's Law.

As mentioned earlier, immune-mediated respiratory effects like asthma may also have relevance for acute hazard, but available studies do not provide sufficient information to characterize dose-response relationships for acute inhalation exposures. Although may be a potential source of uncertainty for the acute POD, dose-response data for these additional respiratory endpoints are used as the basis for the chronic inhalation POD.

4.1.2.2 Chronic Inhalation

OPP and OPPT rely on the chronic inhalation hazard endpoints and PODs derived in the draft IRIS assessment on formaldehyde (U.S. EPA, 2022). The draft IRIS assessment performed dose-response analysis for a range of respiratory and non-respiratory effects to derive a chronic RfC. Endpoints IRIS evaluated for dose-response analysis and considered for POD derivation include sensory irritation, pulmonary function, immune-mediated conditions (asthma and allergy-related conditions), respiratory tract pathology, nervous system effects, and developmental and reproductive toxicity.

Most commonly when deriving a RfC, IRIS selects a critical effect for the endpoint used to derive the POD. In the case of formaldehyde, IRIS chose a suite of impacts to the respiratory system. As described in the draft IRIS assessment, the overall RfC of 0.007 mg/ m^3 was "chosen to reflect an estimate of continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (U.S. EPA, 2022) (pg. 89). IRIS estimated individual RfCs for each organ- or system-specific effect and applied the appropriate uncertainty factors to those individual underlying POD values. In the draft IRIS assessment, this resulted in candidate chronic non-cancer toxicity values of 0.006 to 0.009 mg/m^3 for the highest confidence dose-response datasets, based on effects on the respiratory system (*i.e.*, sensory irritation, pulmonary function, allergy related conditions, and current asthma prevalence or degree of control). EPA acknowledges that comments were made on one of the residential studies (Hanrahan et al., 1984), both from the HSRB in their review of a draft derivation of an acute inhalation toxicity value (HSRB, 2023a) as well as by NASEM in their review of the draft IRIS chronic RfC (NASEM, 2023). OPP and OPPT have been in contact with the IRIS program regarding potential revisions to the chronic RfC based on these comments. Considering concerns raised by peer reviewers, IRIS now interprets the POD derived from this study with a lower level of confidence, thus reducing its utility in supporting a chronic non-cancer inhalation toxicity value given the other available higher confidence datasets for POD derivations. Accordingly, IRIS plans to revise the candidate chronic non-cancer toxicity values selected to support the RfC (*i.e.*, from selected values of 0.006, 0.007, 0.008, and 0.009 mg/m^3 in the external review draft to selected values of 0.006, 0.007, and 0.008 mg/m^3) to reflect the highest confidence datasets for dose-response analysis. Thus, the updated consideration of Hanrahan had minimal impact and this revision does not impact the overall RfC selected by IRIS. In the future, any relevant revisions

being made to the IRIS assessment for NASEM comments will be incorporated into the OPP and OPPT evaluations as appropriate.

IRIS selected the overall RfC of 0.007 mg/m^3 based on the midpoint of the highest confidence candidate values (see Section 2.1.4 of the external review draft IRIS assessment). Uncertainty factors are embedded in the calculation of each candidate toxicity value supporting the RfC. Because OPP and OPPT estimate inhalation risk by calculating margins of exposure (MOE) with a POD that are compared to levels of concern derived from UFs in order to identify any risks of concern, they will rely on the conclusions in the draft IRIS assessment and use the POD cited in the draft IRIS Table 2-3, that is, 0.017 ppm or 0.021 mg/m^3 from Krzyzanowski et al. (1990) and its attendant total UF of 3. For risk assessment purposes, this is quantitatively equivalent to using the draft IRIS RfC value of 0.007 mg/m^3.

Chronic POD= 0.017 ppm (0.021 mg/m^3)
UF= 3× (UF_H = 3)

4.2 Dermal

4.2.1 Summary of Hazard Endpoints

OPP and OPPT identified both human and animal data on the effects of dermal formaldehyde exposure. Many of the available studies have uncertainties related to the purity and stability of formaldehyde treatments. Most commercially available aqueous formaldehyde contains methanol as a stabilizer. In the absence of a methanol control, some of the effects observed following dermal treatments with these aqueous solutions may not be decisively attributed to formaldehyde on its own. Because methanol is not a dermal sensitizer (ECHA, 2024), it is not expected to contribute to sensitization observed in these studies. However, the potential for methanol to increase dermal absorption for formaldehyde is a source of uncertainty in these studies.

Skin Irritation

Several studies in humans and animals show that dermal exposure to formaldehyde can cause skin irritation. Two observational epidemiologic studies investigated the association between formaldehyde dermal exposure to formaldehyde in air and associated health outcomes. These two studies (Socie et al., 1997; Kilburn et al., 1985) conducted questionnaire surveys and included job titles and intensity frequency to estimate dermal formaldehyde exposure for fiberglass batt makers (phenol-formaldehyde-plastic foam matrix embedding of fiberglass), histology technicians, and plastic industry workers. In the Kilburn et al., (1985) study, all studied populations were men, and they showed that fiberglass batt makers and histology technicians had dermal symptoms such as cracking, tightening, peeling, blistering, and pain. Batt makers who were exposed to fiberglass had itching, drying, and burning skin symptoms more frequently. With greater exposure to formaldehyde, the studied population had increasing skin symptoms like thickening, hair loss, nail changes, and boils. Socie et al., (1997) studied plastic industry workers, and most of them were male. This study used a self-administrated questionnaire and self-determined diseases (dermatitis, eczema, red-inflamed, and skin rash) to evaluate the odds ratios. It found that the female population had a higher odds ratio than men. Because these are self-reported observational studies, the underlying cause of these skin reactions is unknown.

Animal studies have indicated that dermal formaldehyde exposure may induce skin irritation, though effects are not consistent across studies. In rabbits, focal areas of edema, abraded, and raised skin were reported 4, 24, and 72 hours following initial exposure to 0.5 mL of formaldehyde to rabbits' backs in two sites (IBT Labs, 1972). However, an 8 week-long chemical patch test on the New Zealand White/Albino Rabbit suggested that formaldehyde had low irritancy potential based on a 0.9728 ±

0.2332 coefficient of irritancy compared to the −4.1459 ± 0.4364 co-efficient of irritancy for water (Nethercott et al., 1984).

Skin Sensitization and Other Immune Effects

Formaldehyde is a known dermal sensitizer in humans. Dermal sensitization, or allergic contact dermatitis, is a Type 4 or delayed-type cell-mediated immune reaction. It is a T-cell mediated inflammation of the skin caused by repeated exposure to antigens (haptens) in a sensitized individual. It occurs in two phases: induction and elicitation. During the induction phase, sensitization of the T cells to the antigen occurs in the draining lymph nodes (Scott et al., 2002). The subsequent elicitation phase is initiated by additional contact with the antigens and is characterized by severe dermal inflammation, erythema, and edema.

Numerous intentional dosing studies have tested people for formaldehyde allergies using patch tests (skin testing systems designed to identify human allergies) at a concentration of 1 or 2 percent, often in a clinical setting where positive results are seen at varying rates. These studies also include investigations of the rates of positive patch tests in professionals with potentially higher exposure to formaldehyde, including health care professionals, hairdressers, and metal workers. Other human intentional dosing studies are available that test at lower concentrations in an attempt to establish minimum elicitation thresholds for skin sensitization (Flyvholm et al., 1997; Fischer et al., 1995).

In animals, there is evidence that dermal exposure to formaldehyde induces an immune response. For instance, Kwak et al., (2014) evaluated the effect of either 4 percent formaldehyde or acetone olive oil spread vehicle repeatedly on the dorsum of the ear of 8-week-old IL-4/Luc/CNS-1 Tg mice for two weeks. At the end of the exposure, mice were imaged for bioluminescence (measuring IL-4 via luciferase signaling assay), weighed for body weight, several tissues/organs (ear, thymus, spleen, heart, etc.) were collected for histopathology, serum was extracted to measure IgE and IL-6, while VEGF proteins were measured in the ear tissue. Results indicate that formaldehyde increased serum IgE concentrations (Type-I hypersensitivity reaction), inflammatory and mast cells (via histopathology), IL-6 and VEGF protein expression, and overall increased epidermis and dermis thickness compared to control. Additionally, both Usuda et al., (2012) and Saito et al., (2011) revealed that dermal exposure of 5 percent formaldehyde solution primarily induces ear swelling and thickness in a TRPV1 KO mouse model study.

Several studies in animals indicate that dermal formaldehyde exposure induces skin sensitization. Studies in guinea pigs indicate that dermal formaldehyde exposure induces skin sensitization and histopathology as seen through the guinea pig maximization test, the Buehler test, split adjuvant technique, guinea pig optimization test, Guillot/Brulos test, Freund's complete adjuvant test, Dossou and Sicard's method, and the open epicutaneous test (Lee et al., 1984; Guillot et al., 1983). Formaldehyde also induced allergic responses such as dermal edema and erythema. In skin patch tests in dogs, formalin induced moderate to intense erythema in 2 of the 3 dogs tested via an open epicutaneous test (Hayasaki and Hattori, 2000). Lastly, in an LLNA assay in 6- to 12-week-old CBA/Ca mice, formaldehyde application to the ear increased their stimulation index (SI) as demonstrated by an increased EC3 value (the concentration required to induce a SI of 3 relative to the concurrent vehicle control) (Basketter et al., 2003). These results indicate that formaldehyde induces skin sensitization in several animal models.

Other animal studies report scarring, swelling, or changes in skin thickness following dermal formaldehyde exposure. A dermal study in rabbits revealed that 0.25 percent formalin did not alter inflammatory cell infiltration but did increase scar tissue formation and density of vascular proliferation. Eight-week-old IL-4/Luc/CNS-1 Tg mice that were exposed to 4 percent formaldehyde dissolved in

acetone olive oil for 2 weeks developed increased ear and ear vein outline thickness (Kwak et al., 2014). Another mouse study conducted with C57Bl/6, BALB/C, and TRPV1 KO mice indicated that formaldehyde induces skin histopathological effects including ear swelling, infiltration of inflammatory cells and hypertrophy of the epidermis in wildtype animals treated with 5 percent formaldehyde, whereas the KO mice had similar effects, but were milder (Usuda et al., 2012). Moreover, guinea pigs exposed to 4 percent formaldehyde for 10 days developed significant skin-fold thickness when compared to pre-treatment levels after exposure period (Wahlberg, 1993).

In addition to human and animal skin sensitization data, multiple, validated non-animal tests are available that are mechanistically associated with key events in the adverse outcome pathway (AOP) for skin sensitization (Strickland et al., 2018). The AOP for skin sensitization initiated by covalent binding to proteins is described by OECD (2014). The AOP for skin sensitization is initiated by key event 1 (KE1), which is followed sequentially by three KEs with well-accepted biological significance: (KE2) keratinocyte activation, (KE3) dendritic cell activation, and (KE4) proliferation of antigen-specific T cells. Several non-animal methods with internationally recognized test guidelines adopted by OECD member countries (including the EPA) assess the ability of chemicals to activate the first three KEs (OECD, 2023a, b).

Based on EPA's previous work using *in vitro* data in quantitative risk assessment for skin sensitization (U.S. EPA, 2020), OPP and OPPT reviewed the available OECD guideline *in vitro* data related to formaldehyde. Formaldehyde is discussed in the chemical list in OECD No. 336 (OECD, 2023a) and results are available for the direct peptide reactivity assay (DPRA), KeratinoSens and human Cell Line Activation Test (h-CLAT) *in vitro* assays (OECD, 2023b, c). Formaldehyde is also included in the Hirota at al., (2015) comparative analysis of *in vitro* predicted EC3 values and animal based LLNA studies. The methods and approaches used in this publication were reviewed as part of the recent OPP draft risk assessment for isothiazolinone biocides[4] and are equivalent to the independent ANN analyses performed by NICEATM in support of the risk assessment. According to supplementary information in Hirota et al. (2015), predicted EC3 values for formaldehyde range from 0.34 to 0.52 percent, equivalent to 85 to 130 µg/cm^2. Predicted EC3 values from *in vitro* data for formaldehyde provide another line of evidence for establishing quantitative levels of skin sensitization induction.

Other Endpoints

Animal evidence on other endpoints following dermal formaldehyde exposure is limited. Two cancer studies in mice (Iversen, 1988; Company Withheld, 1984) evaluated but found no effect on a limited set of non-cancer endpoints, including body weight changes, clinical signs, and mortality, following dermal exposure to formaldehyde. Both studies have major limitations that reduce confidence in the results.

OPP and OPPT also identified one dermal exposure developmental study in hamsters. The study did not identify any significant developmental effects of dermal formaldehyde exposure, but had substantial limitations related to uncertainty around the administered dose and concerns about the volatility of formaldehyde, and the limited timing of the exposure duration relative to sensitive windows of development (Overman, 1985).

4.2.2 Identification of Endpoints for Dose-Response and POD Derivation

Based on available human and animal data, OPP and OPPT identified sensitization as the key endpoint for dermal POD derivation. Formaldehyde is a known dermal sensitizer. For this assessment for formaldehyde, skin sensitization was determined to be the most sensitive non-cancer effect of dermal

[4] See Federal Register Notice.

exposure for which data are available. An approach to quantifying risk from exposure to products containing dermal sensitizing pesticide chemicals that do not bear labels was developed by EPA for assessment of risk from exposure to treated wood (U.S. EPA, 2004). For the isothiazolinone biocides, OPP also used a quantitative approach to assess the risk to isothiazolinone biocides for skin sensitization (U.S. EPA, 2020) utilizing both *in vitro* data and *in vivo* human and animal studies. These previous assessments provide a model for POD derivation based on sensitization from formaldehyde exposure presented below.

Two human patch test studies (Flyvholm et al., 1997; Fischer et al., 1995) investigated elicitation responses to formaldehyde in sensitive individuals. EPA consulted with the HSRB on its scientific and ethical reviews of these two studies in October 2023 (HSRB, 2023b). The HSRB agreed with the EPA's assessment that these studies were scientifically sound and ethically conducted for use in establishing a POD for formaldehyde skin sensitization when considered with other available data. The feedback from the HSRB was incorporated into the final DERs prepared for each study and is reflected in the discussion below.

In Flyvholm et al., (1997), the authors investigated the eliciting threshold concentration of formaldehyde in formaldehyde-sensitive individuals in occluded and non-occluded patch tests and evaluated the relationship to a repeated open application test (ROAT) with a product containing a formaldehyde releaser. Twenty formaldehyde-sensitive individuals agreed to participate in the study, and the control group consisted of 20 healthy volunteers with negative patch tests to formaldehyde. Occluded (0, 25, 50, 250, 500, 1000, 5000, and 10,000 ppm) and non-occluded (0, 25, 50, 100, 250, 500, 1000, and 5000 ppm) patch tests were conducted with formaldehyde solutions in concentrations equivalent to 0, 0.0025, 0.0050, 0.010, 0.025, 0.050, 0.1, 0.5, and 1 percent and ROAT for 1 week with a leave-on cosmetic product containing on average 300 ppm (equivalent to 0.03%) formaldehyde, were carried out simultaneously on each subject. The area of skin treated for the occluded test was 0.5 cm^2 (based on 0.8 mm diameter Finn chamber), the non-occluded test was 1 cm^2, and the ROAT was a 5 by 5 cm area. In the occluded patch test, 19 of the 20 formaldehyde-sensitive subjects reacted to 10,000 ppm formaldehyde, 9 reacted to 5,000 ppm, 3 reacted to 1,000 ppm, 2 reacted to 500 ppm, and 1 reacted to 250 ppm. A LOAEL value of 250 ppm (equivalent to 0.025% or 7.5 $\mu g/cm^2$) and a NOAEL value of 50 ppm (equivalent to 0.005% or 1.5 $\mu g/cm^2$) were established from this study. The HSRB (2023b) agreed with the EPA's assessment that the study could be used as part of endpoint selection and derivation of a POD for elicitation of dermal sensitization. The HSRB also agreed that the study was ethically conducted.

In a study by Fischer et al., (1995), the dose response of the TRUE Test™ system (a novel "dry" test system developed for formaldehyde skin testing) was compared to standard formaldehyde patch tests in aqueous solution (Finn Chamber system) in a series of tests with a range of concentrations for formaldehyde-sensitive individuals. Five different groups were utilized to determine levels at which irritation versus sensitivity occur, as well as a comparison of positive reactions to the TRUE Test system compared to aqueous formaldehyde patch tests at a range of test concentrations. OPP and OPPT focused on Group 2, where a dilution series was tested with both the TRUE Test and formaldehyde 1 percent aqueous patch test systems in formaldehyde-sensitive subjects. Testing on formaldehyde sensitive individuals for each system was conducted at 0.02, 0.03, 0.04, 0.08, 0.12 and 0.15 mg/cm^2 for the TRUE Test system and at 0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1.0 percent (equivalent to 0.0045, 0.0096, 0.019, 0.039, 0.075, 0.15 and 0.3 mg/cm^2) in the Finn Chamber system. The lowest dose for positive reaction from the Finn Chamber was 0.015 percent (equivalent to 0.0045 mg/cm^2 or 4.5 $\mu g/cm^2$) versus 0.01 mg/cm^2 (equivalent to 10 $\mu g/cm^2$) from the TRUE Test system, reflecting the lowest concentration tested for each system. The LOAEL value from this study is 0.015 percent (equivalent to 0.0045 mg/cm^2

or 4.5 µg/cm^2); no NOAEL value was established. The HSRB (2023b) recommended that "the data from this study, in particular from the Finn Test used in Group 2, could be used to corroborate results of studies that were specifically designed to identify a formaldehyde dermal sensitization elicitation threshold from dermal exposure" (HSRB, 2023; pg. 14). The HSRB (2023b) agreed that the study was ethically conducted.

OPP and OPPT identified additional intentional dosing human studies through systematic review but will not rely on them to establish a POD. Some of the studies represented less sensitive elicitation threshold values than the studies referenced above and therefore would not impact the selection of the POD. Other human intentional dosing studies tested at lower concentrations but were not informative in the determination of the POD for skin sensitization for various reasons including: limited or no data on the quantitative analytical methods, no dose provided for skin loading (in the units used in the risk assessment for exposure) or limited study participant information. Most intentional dosing studies identified in the systematic review process involved testing for formaldehyde allergies using patch tests at a concentration of 1 or 2 percent, often in a clinical setting. Numerous studies were identified that tested at this level, generally in individuals not previously sensitized to formaldehyde. OPP and OPPT is not relying on any intentional dosing studies other than the Flyvholm and Fisher studies discussed above; however, for purposes of completeness, the additional intentional exposure studies identified during systematic review may be found in the *Systematic Review Protocol for the Draft Risk Evaluation for Formaldehyde* (U.S. EPA, 2023).

In a relative allergenic potency study using the local lymph node assay (LLNA) (Basketter et al., 2003), 6 to 10-week-old female CBA/Ca mice (4 animals/group) were dosed with 25 µL formaldehyde (38% aqueous purchased from Sigma and while not specified by the author, current Sigma literature indicates stabilization of aqueous formaldehyde solutions with 10 to 15% methanol[5]) in acetone:olive oil 4:1 (AOO) or in propylene glycol (PG) at concentrations of 0, 0.095, 0.19, 0.38, 0.95, 1.9 percent in AOO or 0, 0.38, 0.95, 1.9, 3.8, 9.5, 19 percent in PG for 3 days. Five days after the first treatment, mice were injected with 250 µL phosphate buffered saline containing 20 µCi of [^{3}H] methyl thymidine (^{3}HTdR) and sacrificed 5 hours later. Draining lymph nodes were collected and pooled from each group of four mice. A stimulation index (SI) was derived by dividing the mean disintegrations per minute (dpm)/node in the test group by that in the vehicle control. Using linear interpolation, the EC3 value was determined. Increased cell proliferation was seen with increasing concentration. Formaldehyde response was stronger in AOO than PG, as demonstrated by the EC3 value of 0.4 percent in AOO vs. 3.6 percent in PG.

As discussed above, additional *in vitro* data is also available for formaldehyde for dermal sensitization. Based on the review of the OECD data and isothiazolone draft risk assessment (U.S. EPA, 2020), for *in vitro* data related to formaldehyde, EC3 values were identified from Hirota et al., (2015). In this study, predicted EC3 values for formaldehyde range from 0.34 to 0.52 percent, equivalent to 85 to 130 µg/cm^2. The methods and approaches used in this publication were reviewed as part of the 2020 isothiazolinone draft risk assessment and are equivalent to the independent ANN analyses performed by NICEATM in support of the risk assessment.

POD Derivation

Considering the data from the human patch studies from Flyvholm and Fischer, the reported NOAEL from Flyvholm et al., (1997) was 50 ppm (equivalent to 1.5 µg/cm^2) (LOAEL = 250 ppm, equivalent to 7.5 µg/cm^2) and the LOAEL from Fischer et al. was 0.015 percent (equivalent to 4.5 µg/cm^2). However,

[5] https://www.sigmaaldrich.com/US/en/product/sial/252549.

based on feedback from the HSRB citing concern with using 1 individual for endpoint determination, Benchmark Dose (BMD version 3.3.2) analysis was conducted using the Flyvholm and Fischer studies, with a Benchmark Response (BMR) of 10 percent, which generated endpoints ranging from 5.9 to 10.5 µg/cm^2 (see Appendix B for details of the BMD analysis). Based on the available animal LLNA data in Basketter et al, (2003), an EC3 value of 0.4 percent (equivalent to 100 µg/cm^2) was observed. In Hirota et al., (2015), using non-animal testing methodologies applied by OPP in the isothiazolone draft risk assessment (U.S. EPA, 2020), predicted EC3 values were generated for a suite of chemicals, including formaldehyde. Generated predictive EC3 values ranged from 0.34 to 0.52 percent, equivalent to 85 and 130 µg/cm^2. A summary of the studies considered for POD derivation is provided in Table 4-3 below.

Table 4-3. Summary of Studies Selected to Contribute to POD Derivation

Citation	Exposure Concentrations (Relevant to POD)	Effect
Flyvholm et al., (1997) Human occluded and non-occluded patch test and ROAT	Human occluded patch test: 0, 25, 50, 250, 500, 1,000, 5,000, and 10,000 ppm (0, 0.0025, 0.0050, 0.010, 0.025, 0.050, 0.1, 0.5, and 1% or equivalent to 0, 0.75,1.5, 7.5, 15, 30, 150 and 300 µg/cm^2)	NOAEL = 50 ppm (equivalent to 0.005% or 1.5 µg/cm^2) LOAEL = 250 ppm (equivalent to 0.025% or 7.5 µg/cm^2) based on positive reaction [a]
Fischer et al., (1995) Human occluded patch testing	Human occluded patch test (Finn Chamber): 0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1.0% (equivalent to 4.5, 9.6, 19, 39, 75, 150 and 300 µg/cm^2)	NOAEL (based on Finn Chamber patch test) not established LOAEL = 0.015% (equivalent to 0.0045 mg/cm2 or 4.5 µg/cm^2); based on positive reaction [a]
Basketter et al., (2003) Local Lymph Node Assay (LLNA)	Acetone in olive oil: 0, 0.095, 0.19, 0.38, 0.95, and 1.9% Propylene Glycol: 0, 0.38, 0.95, 1.9, 3.8, 9.5, and 19%	EC3 = 0.4% in AOO/3.6% in PG (equivalent to 100 µg/cm^2 in AOO and 700 µg/cm^2 in PG) [b]
Hirota et al., (2015) Artificial neural network (ANN) prediction models	N/A	EC3 (range) = 0.34 to 0.52%, (equivalent to 85 to 130 µg/cm^2) [b]

[a] Positive reactions graded from + to ++++ according to International Contact Dermatitis Research Group (ICDRG); skin changes observed may include erythema, edema, infiltration, papules and/or vesicles
[b] EC3 (µg/cm^2) = [EC3 (%) × 25 µL × 10 µg/µL] / 1 cm^2

Based on these data, candidate POD values are outlined below in Table 4-4. Looking across the multiple lines of evidence based on human and animal *in vivo* data, as well as *in vitro* data, the PODs are supportive across studies with consistent effect levels across studies and reflect the expected relationship between elicitation and induction thresholds, which are both represented in the POD values displayed below. The Flyvholm et al. (1997) and Fischer et al. (1995) studies with formaldehyde-sensitive individuals represent elicitation thresholds, whereas the animal and *in vitro* data are representative of induction thresholds. The use of induction threshold values is protective of persons not yet exposed to formaldehyde, while the use of elicitation threshold values is protective of those persons already sensitized to formaldehyde. The exact quantitative relationship between the induction and elicitation threshold for any individual chemical is not known; however, it is generally expected that elicitation

thresholds will be lower than the induction thresholds (Scott et al., 2002). This is reflected in the greater induction threshold of 100 µg/cm^2 for formaldehyde, compared to the elicitation threshold of 10.5 µg/cm^2. Elicitation thresholds from the human study result in a lower uncertainty factor (UF of 10) than the uncertainty factor applied to the induction threshold values based on the use of available animal and *in vitro* data (UF of 100).

Table 4-4. Candidate Acute Dermal PODs Based on Skin Sensitization

Sensitization Phase	Citation	POD Type	Candidate POD Value (µg/cm^2)	UFs
Elicitation	Flyvholm et al. (1997) human occluded patch test only	$BMDL_{10}$	10.5	10 (UF_H = 10)
	Fischer et al. (1995) human occluded patch test only	$BMDL_{10}$	5.9	10 (UF_H = 10)
Induction	Basketter et al. (2003) LLNA study in CBA/Ca mice; AOO vehicle	EC3	100	100 (UF_H = 10, UF_A = 10)
	Hirota et al. (2015) *in vitro* battery	Predicted EC3 range	85–130	100 (UF_H = 10, UF_A = 10)

Based on available data, OPP and OPPT selected an elicitation threshold of 10.5 µg/cm^2 based on BMD analyses (BMR = 10%) conducted using data from Flyholm et al, (1997) as supported by data from Fischer et al., (1995). OPP and OPPT selected an induction threshold of 100 µg/cm^2 based on the LLNA study in mice by Basketter et al., (2003) and as supported by *in vitro* analyses conducted in Hirota et al., (2015), and supporting non-animal sensitization tests reported by OECD.

Elicitation POD= 10.5 µg/cm^2
UF = 10× (UF_H = 10)
Induction POD= 100 µg/cm^2
UF = 100× (UF_A =10, UF_H = 10)

The selected PODs are supported by the following:

- Elicitation POD
  - Consistent with NOAEL and LOAEL from Flyvholm et al., (1997) and Fischer et al., (1995)
  - Responsive to HSRB comments to consider PODs that are not based on 1 individual and consider BMD analyses that combine data across studies
  - Supported as lower value than induction thresholds based on both animal and predicted EC3 values
- Induction POD
  - Consistent with multiple available LLNA animal studies
  - Consistent with predicted EC3 values from *in vitro* data

Sources of Confidence and Uncertainties

The dermal POD is derived from an extensive dataset on dermal sensitization in human, animal, and *in vitro* studies. Multiple streams of evidence from studies evaluating elicitation thresholds in sensitive people and induction thresholds in animal and in *in vitro* assays arrive at similar effect levels. While there are some uncertainties associated with the human studies related to lack of clarity in methods and

data reporting, the concordance in effect levels across multiple streams of evidence increases confidence in the POD.

Most of the available human and animal studies on formaldehyde considered by OPP and OPPT in setting a POD are known or suspected to contain methanol. Because methanol itself is not a dermal sensitizer (ECHA, 2024) methanol is not expected to confound results of dermal sensitization studies in the way it may confound other endpoints. However, it is possible that methanol or other vehicles could increase dermal absorption or otherwise influence the effect of formaldehyde. The potential impact of vehicles like methanol in these studies is a source of uncertainty.

Dermal sensitization is highly variable across individuals. Both the induction and elicitation phases of dermal sensitization are influenced by a number of factors, including application method, vehicle, number, timing, sex, and duration of exposures (OECD, 2021; Scott et al., 2002). Evidence has shown that as the sensitization dose is increased, the concentration required to elicit a challenge response was decreased and vice versa (Scott et al., 2002). While the Flyvholm study evaluates responses in sensitive individuals, the sample size is limited and may not reflect the full range of human responses. A 10× UF_H is used to account for uncertainty related to intraindividual variability.

Dermal sensitization is a sensitive systemic immune endpoint supported by a robust dataset, but there is very limited information on the effect of dermal formaldehyde exposure on other systemic endpoints. For example, a single developmental toxicity study in hamsters reported no effect of dermal formaldehyde treatment on developmental outcomes but also had important uncertainties that limit confidence in the results. Although lack of data on reproductive and developmental outcomes and other systemic effects following dermal exposure could be perceived as a source of uncertainty, the likelihood of a lower POD based on reproductive and developmental outcomes is low given the biological understanding of dermal sensitization and the reactivity of formaldehyde.

4.3 Oral

4.3.1 Summary of Hazard Endpoints

Animal studies have evaluated the effects of oral formaldehyde exposure on a range of health outcomes, including gastrointestinal, immune, reproductive, developmental, and neurological effects. However, technical challenges in generating stable formaldehyde solutions of sufficient purity for repeated oral exposure contributed to major limitations and uncertainties in most of the available animal studies. Most commercially available aqueous formaldehyde contains methanol as a stabilizer. In the absence of a methanol control, effects observed following treatments with these aqueous solutions may not be decisively attributed to formaldehyde on its own. This is complicated further by the fact that formaldehyde is a metabolite of methanol, and both share a common toxic metabolite, formic acid. While such studies are not informative for characterizing dose-response relationships for pure formaldehyde alone, they can support characterization of health effects associated with formalin, which accounts for a large share of occupational exposures.

Other studies prepare aqueous formaldehyde treatments from paraformaldehyde in the absence of stabilizers, avoiding potential confounding from stabilizers. OPP and OPPT focused its review on oral studies conducted with formaldehyde only (in the absence of methanol). Although this improves confidence that effects observed in the studies are specific to formaldehyde, the potential for reduced stability of formaldehyde treatments may reduce confidence in the actual doses achieved.

Gastrointestinal Effects

OPP and OPPT identified three animal studies that evaluate gastrointestinal effects of oral exposure to formaldehyde in the absence of methanol. Two 2-year drinking water studies (Til et al., 1989; Civo Institute TNO, 1987a) and (Tobe et al., 1989) evaluated the effects of chronic exposure to formaldehyde in rats. Both studies reported lesions in the forestomach and glandular stomach. While these studies represent some of the best available information on chronic hazard from oral exposures to formaldehyde, both studies have limitations due to reductions in drinking water intake in treated animals at the high dose.

A third study (Til et al., 1988) evaluated the gastrointestinal effects following 28 days of drinking water exposure. This study included water-restricted controls to determine the extent to which effects observed in formaldehyde-treated animals may be attributable to dehydration. Formaldehyde treated rats in this study also had increased incidence of gastrointestinal histopathology that was not observed in water-restricted controls, increasing confidence that the effects were due to formaldehyde treatment.

Immune Effects

Three animal studies evaluated the effects of oral formaldehyde exposure on immune endpoints. All three studies have major limitations related to the suspected presence of methanol in commercially sourced aqueous formaldehyde used in the treatments in the absence of a methanol control. In addition, all three studies provide limited information on the frequency or preparation of the test substance which contributes to uncertainty about the doses achieved in these studies.

Oral gavage exposure to 20, 40 or 80 mg/kg-day formaldehyde was associated with a dose-dependent reduction in antibody responses and increase in relative lymph node weights in a 28-day study in rats (Vargova et al., 1993). A similar effect level was reported in a single dose study in mice by Abd-Elhakim, (2016). Oral gavage exposure to 25 mg/kg-day formaldehyde for 60 days was associated with spleen histopathology and alterations in hematological parameters (including decreased red blood cells and hemoglobin, increased mean corpuscular hemoglobin concentration, increased packed cell volume, decreased total WBC, lymphocyte and basophile levels, decreased WBC phagocytosis and lysosome activity, decreased IgG levels, and increased IgM levels) (Abd-Elhakim et al., 2016). The third study (Merzoug and Toumi, 2017) reported maternal effects on hematology parameters following 2 mg/kg-day oral gavage exposure to formaldehyde during pregnancy. However, the lack of methanol control in these studies makes it difficult to determine whether reported immune effects are the result of exposure to formaldehyde alone.

Reproductive and Developmental Effects

Several oral exposure studies in animals have evaluated developmental effects of formaldehyde. However, these studies have limitations due to questions of stability of formaldehyde in dietary and drinking water treatments and/or the known or likely presence of methanol, which is commonly used to stabilize formalin and may contribute to observed developmental effects. Oral gavage exposure to 2 mg/kg-day formaldehyde (in the form of a 37% formaldehyde) in rats throughout gestation (prior to mating through GD19) was associated with decreased number of live pups per litter and fetal weight, as well as significant decreases in maternal body weight gain, altered maternal neurobehavioral tests, and changes in maternal hematological parameters and hormone levels (Merzoug and Toumi, 2017). Several studies in mice found no effect of gestational oral gavage formaldehyde exposure on pup survival or pup weight (RTI, 1992; Seidenberg et al., 1986; Marks et al., 1980). A dietary exposure study in dogs also found no effect of dietary exposure to formaldehyde throughout gestation on pup body weight or length of gestation (Hurni and Ohder, 1973).

Two oral exposure studies evaluated the effects of formaldehyde on male fertility. In 9- to 10-week-old male rats, a single oral gavage exposure to 200 mg/kg-day formaldehyde was associated with an increased percentage of abnormal sperm heads (Cassidy et al., 1983) (also described in an unpublished study report (Shell Research, 1982)). Similarly, in adult male mice, oral gavage exposure to 25 mg/kg-day formaldehyde was associated with decreased sperm concentration and motility, increased sperm abnormalities, and histopathological evidence of altered spermatogenesis (Khalil et al., 2017). However, in both studies the known or presumed presence of methanol in the treatment and the lack of a methanol control makes it unclear whether effects reported in these studies are attributable to formaldehyde alone.

Neurological Effects

Several animal studies evaluated neurological endpoints following formaldehyde exposure (Merzoug and Toumi, 2017; Bhatt and Panchal, 1997, 1992), but all were rated uninformative due to uncertainty related to the stability and purity of formaldehyde, the lack of appropriate controls and/or lack of clarity in reporting of study design and results. One study reported altered neurobehavioral tests in female rats exposed to formaldehyde via oral gavage throughout gestation, but the study did not include a methanol control (Merzoug and Toumi, 2017). Another study reported decreased/delayed behavioral performance in rats exposed via drinking water, but there is uncertainty around the stability of formaldehyde in drinking water (Bhatt and Panchal, 1997).

4.3.2 Identification of Endpoints for Dose-Response and POD Derivation

Gastrointestinal effects were found to be the most sensitive endpoint evaluated in the set of studies that were not confounded by methanol.

In one of these studies (Til et al., 1989; Civo Institute TNO, 1987a) Wistar rats (n = 70/sex/group) were exposed to formaldehyde in drinking water at target dose levels of 0, 5, 25, 125 mg/kg-day for 2 years and were evaluated for a range of both cancer and non-cancer effects. Estimated doses adjusted for drinking water intake and stability were 0, 1.2, 15, and 82 mg/kg-day in males and 0, 1.8, 21, and 109 mg/kg-day in females, respectively (based on adjustments for recovery of 35, 89 and 100 percent of low, mid, and high dose, respectively). At the high dose, formaldehyde exposure was associated with "severe damage" to the gastrointestinal mucosa, including raised or thickened limiting ridge and significantly increased incidence of surface lesions in forestomach (including papillary epithelial hyperplasia, hyperkeratosis, and focal ulceration) and/or glandular stomach (including chronic atrophic gastritis, ulceration and/or glandular hyperplasia). High dose animals also had a significant 40 percent decrease in drinking water intake. Reductions in body weight and food intake were also reported.

In the OPPT systematic review process, all health outcomes in this study received a data quality rating of "uninformative," due to a metric-rating of "uninformative" for confounding/variable control as a consequence of the reduced water intake in the high dose group (the cutoff is ≥20% decrease in drinking water intake) and lack of control for decreased water consumption over the 2-year test period as described in the *Systematic Review Protocol for the Draft Risk Evaluation for Formaldehyde* (U.S. EPA, 2023). While the uninformative rating for that metric is consistent with predetermined criteria, there is no specific evidence that dehydration is a confounder for GI histopathology. A recent paper suggests that while dehydration can initiate injury pathways in certain organs, dehydration alone does not result in histopathologic organ phenotypes (Schreurs et al., 2023). In addition to the concern about confounding effects of decreased water intake on interpretation of effects, OPPT's systematic review data quality evaluation noted lower stability of formaldehyde at the low dose in the 2-year study by Til et. al. (Til et al., 1989; Civo Institute TNO, 1987a), reducing confidence in the doses achieved in the low dose group, but not in the middle and high dose groups. This led to a metric rating of low for preparation and storage of test substance.

In the DER, the study was ultimately classified as acceptable/non-guideline following further evaluation. OPP evaluated the stability analysis included in the unpublished report for the 2-year study by Til et. al, (Civo Institute TNO, 1987a) and determined that the study results using the mid-dose (15 mg/kg-day for males and 21 mg/kg-day for females) and high dose (82 mg/kg-day for males and 109 mg/kg-day for females), adjusted for drinking water intake and stability are acceptable for use in formaldehyde hazard characterization.

A 28-day drinking water study (Til et al., 1988) was initiated by the same lab after the start of the two-year study. This study evaluated the same gastrointestinal effects of formaldehyde in Wistar rats (n = 10/sex/group) at the same target dose levels (0, 5, 25, 125 mg/kg-day) and included water-restricted controls, which controlled for the amount of water consumed by the high dose groups. OPP and OPPT adjusted for drinking water intake and stability in this study, estimating that actual doses were 0, 2.1, 26, 130 mg/kg-day in males and 0, 2.1, 25, 135 mg/kg-day in females, respectively (based on adjustments for recovery of 35, 89, and 100% of low, mid, and high dose, respectively, presented in the Til 2-year study recovery analysis). In the 28-day study, the high dose groups and matched water-restricted controls consumed 25 to 30 percent less water compared to unrestricted controls. These decreases were slightly less than the decrease of 40 percent in water intake at the same dose in the 2-year study. This study reported gastrointestinal effects in the high dose groups similar to the findings in the chronic study, including thickening of the limiting ridge, hyperkeratosis of the forestomach, and focal gastritis in the glandular stomach. It is important to note that these effects were not observed in the water restricted controls in this study, consistent with the interpretation that the gastrointestinal effects in this study were treatment-related. In the OPPT systematic review process, this study received a data quality rating of "high" for all health outcomes (U.S. EPA, 2023). Similarly, this study was classified as acceptable/non-guideline in the DER.

In a third study from a different lab (Tobe et al., 1989), Wistar rats (n =20/sex/group) were exposed to formaldehyde through drinking water (0, 10, 50, 300 mg/kg-day) over 2 years. In the high dose group, all rats died by the end of the study. Consistent with the findings in the Til et al studies (Til et al., 1989; Til et al., 1988; Civo Institute TNO, 1987a), there were significant increases in lesions in the forestomach (including squamous cell hyperplasia, hyperkeratosis, and basal cell hyperplasia) and glandular stomach (including glandular hyperplasia and erosion/ulcers) at the high dose, with marginal or equivocal effects on the stomach at the mid-dose. In the OPPT systematic review process, this study received a data quality rating of "uninformative" for all health outcomes, primarily due to potential confounding from reduced water intake and high mortality in the high dose group and a lack of information about the stability of the formaldehyde treatments. The DER identified the same limitations around stability and lack of data reporting; however, the study was ultimately classified as acceptable/non-guideline following further evaluation that demonstrated that the gastrointestinal effects were treatment-related, as discussed below.

Taken together, the three drinking water studies demonstrate a consistent pattern of gastrointestinal effects at comparable dose levels. The mid-dose of the two-year Til et al study (Til et al., 1989; Civo Institute TNO, 1987a) 15 mg/kg-day in males, was not confounded by stability issues or by reduced water intake and showed no adverse effects on the GI tract. The mid-dose of the 28-day Til et al study (1988), 25 mg/kg-day, also showed no adverse effects and received a rating of High for the GI tract. The low-dose of (Tobe et al., 1989), 10 mg/kg-day, showed no adverse effects on the GI tract while the mid-dose of 50 mg/kg-day showed some precursor effects. Taken together, the no effect level for the GI tract lies in the range of 15 to 50 mg/kg-day. While limitations in the two chronic drinking water studies resulted in OPPT data quality ratings of "uninformative for dose response" for the individual studies, the body of evidence across all three studies in combination increases the overall confidence in both the

nature of the effects observed and the levels of formaldehyde exposure associated with those effects. Additional drinking water intake controls in the 28-day study (Til et al., 1988), increase confidence that the observed effects across all three studies are due to formaldehyde as opposed to dehydration. Similarly, the stability analysis performed on the two-year Til et al., 1989 study (Til et al., 1989; Civo Institute TNO, 1987a) increases confidence that conditions described in other studies (e.g., drinking water solution frequency of preparation and storage conditions) result in acceptable stability and target doses being achieved.

Consideration of Whether Gastrointestinal Effects are Due to Formaldehyde or Reduced Water Intake

The dramatic reduction in drinking water intake in the high dose groups (noted in both chronic studies), is the primary reason for the uninformative OPPT data quality ratings because dehydration in those animals could have confounded results. However, as demonstrated in the 28-day study (Til et al., 1988), the gastrointestinal effects observed in response to formaldehyde exposure are not observed in water-restricted controls. While the results of the 28-day study cannot be directly extrapolated to the longer duration and increased severity of water restriction in the chronic studies, it does provide evidence that the gastrointestinal effects seen in the histopathology are treatment-related. In addition, as described above, a recent paper suggests that while dehydration can initiate injury pathways in certain organs, dehydration alone does not result in histopathologic organ phenotypes (Schreurs et al., 2023).

Consideration of Stability

In the absence of a stabilizer such as methanol, the stability of formaldehyde in water becomes a source of uncertainty. The stability analysis performed in the 2-year Til et al. study (Til et al., 1989; Civo Institute TNO, 1987a) helps to define how concentration, frequency of preparation, and other factors can influence stability of formaldehyde solutions. Results of the stability analysis indicate that there is greater stability at higher formaldehyde concentrations and within the first few days in solution; conversely, stability decreases with duration of storage, at higher temperatures, and at lower concentrations in solution. Although experimental data confirmed the lack of stability of formaldehyde at the lowest dose used in the 2-year study by Til et. al, this dose is below the NOAEL for gastrointestinal effects. The experimental data on the stability of the dosing solutions supports that the mid-dose and high-dose were achieved. This supports the identification and reliability of the NOAEL for gastrointestinal effects at the mid-dose. Although the Tobe et al. study (Tobe et al., 1989) does not provide information on the stability of formaldehyde in drinking water prepared for the study, the stability analysis performed by Til et al (Til et al., 1989; Civo Institute TNO, 1987a) demonstrates that while lower concentrations of formaldehyde are less stable in water over time, they appear to be relatively stable in the first 3 days. While these results cannot be directly extrapolated across labs, this increases confidence in the stability of the formaldehyde treatments in the study by Tobe et al (1989) since drinking water solutions were prepared twice weekly, compared to weekly preparation in the 28-day and 2-year studies by Til et. al. Furthermore, the dose levels for which stability is a concern (*e.g.*, most pronounced at 5 mg/kg-day with less decline in concentration at 25 mg/kg-day) are lower than dose levels in the study by Tobe at which marginal/equivocal (50 mg/kg-day) and frank (300 mg/kg-day) treatment-related effects are occurring.

Consideration of Dose-Response across Studies

Examination of the dose-response relationship across studies further increases confidence in the treatment-related effects of formaldehyde on the gastrointestinal tract and the nominal doses at which those effects occur.

- **5 mg/kg-day** (adjusted to 1.2 mg/kg-day in males and 1.8 mg/kg-day in females in the 2-year Til et al. study (Til et al., 1989; Civo Institute TNO, 1987a) – No effects of formaldehyde treatment

at this dose in the 28-day or 2-year studies by Til et. al. Due to stability concerns, the actual achieved dose is inconclusive and is not being considered as part of the dose-response.

- **10 mg/kg-day** – No effects in the 2-year study by Tobe et. al (1989) in which the more frequent preparation (twice weekly) of the treatment solutions imparts greater confidence in the achieved dose.
- **25 mg/kg-day** (adjusted to 15 mg/kg-day in males and 18 mg/kg-day in females in the 2-year Til et al. study (Til et al., 1989; Civo Institute TNO, 1987a)) – No treatment-related effects. Stability analysis indicated that the target mid-dose was achieved when adjusted based on stability and water intake. This is the **NOAEL** for both the 28-day and 2-year studies by Til et. al.
- **50 mg/kg-day** – Only marginal or equivocal effects were observed at the mid-dose in the 2-year study by Tobe et. al (1989), consistent with non-adverse precursor effects to those seen at higher doses, limited to hyperkeratosis of the forestomach in 1/6 males at the 18-month interim sacrifice and in 1/8 females at termination at 24 months.
- **125 mg/kg-day** (adjusted to 82 mg/kg-day in males and 109 mg/kg-day in females in the 2-year Til et al. study (Til et al., 1989; Civo Institute TNO, 1987a)) – This is the **LOAEL** in both the 28-day and 2-year studies by Til et. al. based on treatment-related effects on the stomach, including epithelial hyperplasia; hyperkeratosis, ulceration, atrophic gastritis, and squamous metaplasia. Stability analysis indicated that the target high-dose was achieved when adjusted based on stability and water intake.
- **300 mg/kg-day** – The high dose of 300 mg/kg-day in the 2-year study by Tobe et al. (1989) resulted in 100 percent mortality and severe histopathology findings in the GI tract which were more pronounced with time and compared to lower doses and included incidences of erosions and ulcers in the forestomach and glandular stomach, squamous cell hyperplasia, with and without hyperkeratosis, along with downward growth of basal cells. Mortality occurred as early as 9 days after start of treatment and reaching 45 percent in males and 55 percent in females by 12 months. All females in this dose group were dead by 21 months, and all males were dead by 24 months.

The three oral studies were selected to inform dose-response because they comprise the best available data on oral exposure to formaldehyde for the following reasons: (1) These studies are the only oral studies available which do not include methanol to stabilize the concentration of formaldehyde, which may confound the results; (2) the effects on the gastrointestinal tract can be attributed to formaldehyde and are not confounded by dehydration. OPP and OPPT are not relying on effects seen on other parameters likely confounded by dehydration, such as the decreased body weights and food consumption and changes in urinalysis and clinical chemistry; and (3) OPP and OPPT has confidence in the stability and achieved dose at the NOAEL and LOAEL in the Til et al. 1989 study. OPP and OPPT concluded that, when considered in conjunction with the other two studies, Til et al. 1989 contributes meaningful information to the WOE and dose-response despite the OPPT data quality rating of "uninformative."

Table 4-5. Summary of Studies Selected to Contribute to POD Derivation

Citation	Study type	Effect Level (mg/kg-day)	Effect	Data Quality Summary
Til et al. (1988); Civo Inst. unpublished (1991)	28 days; Oral Drinking Water in Rats (Cpb:Wu; Wistar random) Target intake levels 0, 5, 25, or 125 mg/kg-bw/day Mean doses administered[a]: Males: 0, 2.1, 26, 130 mg/kg-day Females: 0, 2.1, 25, 135 mg/kg-day N=10/dose/sex	NOAEL = 25 LOAEL = 135 based on clinical chemistry and histopathology of the GI tract (fundic thickening, hyperkeratosis of the forestomach, focal gastritis of the glandular stomach).	Gross necropsy observations showed focal fundic thickening, described as "remarkable" in all high-dose animals, with some animals showing yellowish discoloration in the forestomach, hyperkeratosis, moderate papillomatous hyperplasia, and slight focal atrophic gastritis in forestomach.	Includes control group with water restricted to intake amount of highest dose. OPPT data quality rating: *high* OPP DER classification: *acceptable/ non-guideline*
Civo Inst., (1987a) (unpublished); Til et al (1989); Civo Inst., (1987b) 12- month interim kill report corresponding to Til 1989	Chronic: 2 years; Oral Drinking Water in Rats (Cpb:Wu; Wistar random) Target intake levels 0, 5, 25, 125 mg/kg-day. Mean doses administered: Males: 0, 1.2, 15, 82 mg/kg-day Females: 0, 1.8, 21, 109 mg/kg-day	NOAEL = 15 LOAEL = 82 based on GI histopathology	Decreased body weight, water consumption, and food consumption at high dose in both sexes. Stomach: Gross: Limiting ridge of forestomach was raised & thickened; surface lesions in forestomach and/or glandular stomach. Histopath: papillary epithelial hyperplasia, hyperkeratosis, focal ulceration in forestomach, chronic atrophic gastritis; ulceration and/or glandular hyperplasia in glandular stomach. Kidneys: renal papillary necrosis	Palatability issues, substantially reduced drinking water intake, introducing uncertainty around doses achieved and potential confounding of results related to dehydration. OPPT data quality rating: *uninformative for dose-response* OPP DER classification: *acceptable/ non-guideline*

Tobe et al, (1989)	Chronic: 24 months; Oral Drinking Water study in Wistar Rats (0, 10, 50, 300 mg/kg-day) N=20/dose/sex	NOAEL = 10 LOAEL = 50 based on forestomach hyperkeratosis	At 50 mg/kg-day hyperkeratosis of the forestomach in 1/6 males at the 18-month interim sacrifice and in 1/8 females at termination at 24 months. At the highest dose, all animals died by 24 months.	Absolute/relative body and organ weights were not provided. Test substance concentration and lack or reporting results. no data provided on organ, body weight, tumors seen, Test solutions were made up twice weekly using paraformaldehyde. OPPT data quality rating: *uninformative for dose-response* OPP DER classification: *acceptable/ non-guideline*
[a] OPP and OPPT adjusted for drinking water intake and stability in this study, estimating that actual doses were 0, 2.1, 26, 130 mg/kg-day in males and 0, 2.1, 25, 135 mg/kg-day in females, respectively (based on adjustments for recovery of 35, 89, and 100% of low, mid, and high dose, respectively, presented in the Til 2 yr study recovery analysis). The adjusted NOAEL in females is equal to the nominal dose.				

Subchronic POD Derivation

OPP and OPPT selected a subchronic POD of 25 mg/kg-day based on the NOAEL for gastrointestinal histopathology in rats reported following 28 days of formaldehyde exposure through drinking water in Til et al, (1988). This POD is based on dose-response information in a high-quality study with a relevant exposure duration. It is supported by consistent effects in the two chronic drinking water studies.

Subchronic POD = 25 mg/kg-day

Consistent with EPA guidance on deriving an oral HED for portal-of-entry effects (U.S. EPA, 2011), OPP and OPPT applied a dosimetric adjustment factor (DAF) to convert the POD identified in rats to a human equivalent dose (HED) using body weight ¾ allometric scaling. Specifically, the following equation was used:

HED (mg/kg-day) = POD (mg/kg-day) × DAF

where DAF = 0.24 (based on the DAF using bodyweight ¾ scaling from rats to humans reported in Appendix B of US EPA (2011)). An uncertainty factor of 30x was applied to this POD (3x interspecies extrapolation, 10x intraspecies variation). The interspecies uncertainty factor is reduced to 3x based on the application of the DAF which accounts for the pharmacokinetic differences between rats and humans (U.S. EPA, 2011).

Subchronic HED = 6 mg/kg-day

UF = 30× (UF_A = 3, UF_H = 10,)

Chronic POD Derivation

OPP and OPPT considered candidate PODs from each of the three studies, as summarized in Table 4-6. A chronic POD of 15 mg/kg-day was selected based on the NOAEL for gastrointestinal histopathology in rats following 2 years of formaldehyde exposure through drinking water (Til et al., 1989; Civo Institute TNO, 1987a). The selected POD is supported by the NOAEL of 25 mg/kg-day following 28 days of exposure reported in Til et al (1988), identified as a high-quality study. It is further supported by the equivocal/marginal gastrointestinal effects occuring at 50 mg/kg-day reported in Tobe et al (1989) following 2 years of exposure to formaldehyde via drinking water.

Table 4-6. Candidate Chronic Oral PODs Based on Gastrointestinal Histopathology

Citation	Study Type	Candidate POD	Candidate HED	Relevant UFs	Total UF
Til et al. (1988)	28-day drinking water study in rats	25 mg/kg-day	6 mg/kg-day	UF_A = 3 UF_H = 10 UF_S = 10[a]	300
Civo Inst. (1987a) and Til et al. (1989)	2-year drinking water study in rats	15 mg/kg-day	3.6 mg/kg-day	UF_A = 3 UF_H = 10	30
Tobe et al. (1989)	2-year drinking water study in rats	50 mg/kg-day	12 mg/kg-day	UF_A = 3 UF_H = 10	30

[a] OPP and OPPT acknowledge uncertainty around application of the UF_S given the consistency of candidate PODs across study durations and the lack of apparent progression of effects between subchronic and chronic studies.

Concordance across the three studies increases overall confidence in the POD. When considered in isolation, limitations of the Til 1989 study (Til et al., 1989; Civo Institute TNO, 1987a) introduce uncertainties around the nature of the dose-response relationship and the degree to which the effects are due to formaldehyde rather than dehydration. However, evidence from the other two other studies increases confidence that formaldehyde exposure causes gastrointestinal effects.

Chronic POD = 15 mg/kg-day

Consistent with EPA guidance on deriving an oral HED for portal-of-entry effects (U.S. EPA, 2011), OPP and OPPT applied a dosimetric adjustment factor (DAF) to convert the POD identified in rats to a human equivalent dose (HED) using bodyweight ¾ scaling. Specifically, the following equation was used:

HED (mg/kg-day) = POD (mg/kg-day) x DAF

where DAF = 0.24 (based on the DAF using bodyweight^{¾} scaling from rats to humans reported in Appendix B of U.S. EPA (2011)). An uncertainty factor of 30× was applied to this POD (3× interspecies extrapolation, 10× intraspecies variation). The interspecies uncertainty factor is reduced to 3x based on the application of the DAF which accounts for the pharmacokinetic differences between rats and humans (U.S. EPA, 2011).

Chronic HED= 3.6 mg/kg-day

UF = 30 (UF_A =3, UF_H = 10,)

Sources of Confidence and Uncertainties

The subchronic and chronic oral PODs rely on a limited database of animal studies but are supported by three studies that report consistent patterns of gastrointestinal damage at similar doses.

Due to technical challenges around generating pure and stable formaldehyde treatments for oral exposure, most of the available animal studies have major limitations and uncertainties. Among the available studies that are not confounded by the presence of methanol, gastrointestinal effects are the most sensitive endpoint evaluated. As described above, reduced drinking water intake in the high dose groups reduced confidence in each of the chronic studies when considered in isolation. The limitations in these studies may reduce their sensitivity to detect effects on other sensitive health outcomes like body weight. However, when considered in conjunction with the results of the 28-day study that included water-restricted controls, OPP and OPPT have confidence that the reported effects are attributable to formaldehyde exposure.

There is very limited information on reproductive, developmental, and immune endpoints following oral exposure to formaldehyde. While there are some studies that suggest effect levels for these endpoints may be more sensitive than those used as the basis for the POD (see Section 4.3.1), the only studies that evaluate immune, reproductive, or developmental endpoints are confounded by the presence of methanol. Evidence of reproductive and developmental effects reported in humans and animals following inhalation exposure to formaldehyde indicates that such effects are possible following formaldehyde exposure. Similarly, the available data do not evaluate factors that may increase susceptibility to oral formaldehyde exposure in sensitive groups or lifestages. The lack of data on these endpoints and sensitive groups and lifestages following oral exposure could be perceived as uncertainty; however, the likelihood of a lower POD being identified based on these outcomes is low given the effect

used as the basis of the current PODs (gastrointestinal effects) are close to the portal of entry, first pass metabolism via the oral route, and the reactivity of formaldehyde.

4.4 Summary of Hazard Values for Formaldehyde

Table 4-7 summarizes the cancer and non-cancer hazard values identified for formaldehyde as described throughout Sections 3 and 4. These hazard values will be used to support risk calculations in OPP and OPPT assessments.

Table 4-7. Toxicological Doses and Endpoints for Formaldehyde for Use in Occupational and Residential Human Health Risk Assessments.

Exposure/Scenario	Hazard Value	Uncertainty Factors	Total Uncertainty Factor	Study and Toxicological Effects
Inhalation Acute	NOAEC = 0.5 ppm (0.62 mg/m^3) as a 15-minute peak exposure	UF_H = 10	Total UF = 10	Kulle et al, (1987) LOAEC = 1 ppm (mg/m^3) based on eye irritation in adult volunteers Mueller et al, (2013) LOAEC = 0.3 ppm over four hours, with 15-minute peaks of 0.6ppm, based on eye irritation in hypersensitive adult volunteers Lang et al, (2008) LOAEC= 0.5 ppm over 4 hours, with peaks of 1 ppm (0.62/1.23 mg/m^3), based on eye irritation in adult volunteers
Inhalation Chronic non-cancer (Long-term, >6 months)	$BMCL_{10}$ = 0.017 ppm (0.021 mg/m^3)	UF_H = 3	Total UF= 3	POD is derived from the draft IRIS RfC (U.S. EPA, 2022). The specific $BMCL_{10}$ value used here is based on reduced pulmonary function in children in Krzyzanowski et al., (1990), but is consistent with the draft RfC derived by IRIS based on multiple studies of respiratory system effects.
Inhalation Chronic Cancer	Adult-based IUR: 0.0079 ppm^{-1} (6.4E−6 (µg/m^3)$^{-1}$) ADAF-adjusted IUR: 0.013 ppm^{-1} (1.1E−05 (µg/m^3)$^{-1}$)	N/A	N/A	IUR presented in the draft IRIS assessment (U.S. EPA, 2022) based on data on nasopharyngeal cancer in people reported in Beane-Freeman et al. (2013)
Dermal Acute	Induction: EC3 = 0.4% v/v (100 µg/cm^2) in 4:1 acetone:olive oil	UF_A = 10 UF_H = 10	Total UF= 100	Basketter *et al.*, (2003) based on induction of dermal sensitization in mice
	Elicitation: $BMDL_{10}$ = 10.5 µg/cm^2 (0.035%)	UF_H = 10	Total UF = 10	Flyvholm, MA. *Et al.* (1997) based on threshold for elicitation of dermal sensitization in people

Exposure/Scenario	Hazard Value	Uncertainty Factors	Total Uncertainty Factor	Study and Toxicological Effects
Oral Short-Term/ subchronic (1–30 days)	HED = 6 mg/kg-day	UF_A = 3 UF_H = 10	Total UF = 30	Til (1988) NOAEL= 25 mg/g-day; LOAEL = 125 mg/kg-day based on gastrointestinal histopathology in rats
Oral Chronic	HED = 3.6 mg/kg-day	UF_A = 3 UF_H = 10	Total UF = 30	Civo Inst.(1987a); Til (1989) NOAEL= 15 mg/g-day; LOAEL = 82 mg/kg-day based on gastrointestinal histopathology in rats

Point of departure (POD) = A data point or an estimated point derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no-observed adverse-effect level. LOAEL = lowest-observed adverse-effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_L = use of a LOAEL to extrapolate a NOAEL. UF_S = use of a short-term study for long-term risk assessment. UF_{DB} = to account for the absence of key data (i.e., lack of a critical study). N/A = not applicable. IUR= inhalation unit risk; ADAF-adjusted IUR = IUR for calculating cancer risks associated with a full lifetime of exposure

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Appendix A Regulatory Limits

Table_Apx A-1 contains exposure limits for acute inhalation exposures to formaldehyde set by other authoritative sources.

Table_Apx A-1. Summary of Acute Inhalation Exposure Limits Set by Other Authoritative Sources

Agency/ Description [a]	Endpoint	Value [b c]	Key Citation(s)	Notes
Exposure limits for residential and general population exposures				
1999 ATSDR acute MRL (<14 days)	Sensory irritation	24-hour TWA = 0.04 ppm	Pazdrak et al. (1993)	Based on sensory irritation (eye and nasal) in intentional human exposure. This MRL incorporates a UF of 9 (3 for use of a LOAEL; 3 for human variability).
2008 AEGL-1	Eye irritation	10-minute STEL = 0.9 ppm	Bender et al. (1983)	Based on irritation in controlled human exposures. The same value was selected for all exposure durations ranging from 10 min to 8 hr.
2008 EPA-OPP RED	Sensory Irritation	Residential RfC = 0.01 ppm	Horvath et al. (1988)	Based on sensory irritation (eye, nasal, and throat) reported in an occupational epidemiological study; the NOAEL of 0.1 ppm was applied for all durations (acute and chronic) applying an intraspecies UF of 10 for residential scenarios.
2021 Health Canada	Sensory irritation	Short Term (1 hr) = 0.1 ppm	Kulle (1993)	The short-term limit (1-hour average) is based on eye, nose, and throat irritation.
2010 WHO Guideline for short-term exposures	Eye irritation	30 min STEL = 0.08 ppm	Lang et al. (2008) Supporting evidence from Kulle et al. (1987)	The NOAEL of 0.6 mg/m^3 (0.5 ppm) for the eye blink response is adjusted using an assessment factor of 5 derived from the standard deviation of nasal pungency (sensory irritation) thresholds, leading to a value of 0.12 mg/m^3, which was rounded down to 0.1 mg/m^3 (0.08 ppm).
Exposure limits for occupational exposure				
2017 ACGIH-TLV	URT and Eye irritation URT Cancer	8 hr TWA = 0.1 ppm 15 min STEL = 0.3 ppm	Lang et al. (2008) Supporting evidence from (Alexandersson and Hedenstierna, 1988; Andersen and Molhave, 1983)	These values are recommended to minimize the potential for sensory irritation, chiefly of the eye and upper respiratory tract (URT). The LOAELs for eye and URT irritation from human experimental studies (Lang, 2008) and cross-sectional studies of workers (Alexandersson and Hedenstierna, 1988) involved both continuous and peak exposures.
2008 EPA-OPP RED	Sensory Irritation	Occupational RfC = 0.1 ppm [d]	Horvath et al. (1988)	Based on sensory irritation (eye, nasal, and throat) reported in an occupational epidemiological study; the NOAEL of 0.1 ppm was applied for all durations (acute and chronic) applying a total UF of 1.
1992 OSHA	URT and eye irritation URT Cancer	8 hr TWA = 0.75 ppm 15 min STEL = 2 ppm	FR Doc 92-11911	The OSHA PEL and STEL were established in 1987 and revised in 1992. They represent a compromise between human health and feasibility.

Agency/ Description [a]	Endpoint	Value [b c]	Key Citation(s)	Notes
1986 NIOSH	URT and eye irritation URT Cancer	8 hr TWA = 0.016 ppm 15 min STEL = 0.1 ppm	Unknown	The NIOSH REL and STEL were established in 1986 and have not been updated since. They only consider human health.
2016 EU SCOEL	Sensory irritation	8 hr TWA = 0.3 ppm 15 min STEL = 0.6 ppm	Lang et al. (2008) Mueller et al. (2013)	Based on eye and URT irritation. No uncertainty factors applied.

[a] ATSDR = Agency for Toxic Substances and Disease Registry; AEGL = acute exposure guideline levels for airborne chemicals; RED = Re-registration Eligibility Decision; WHO = World Health Organization; ACGIH-TLV = American Conference of Governmental Industrial Hygienists-Threshold Limit Value; OSHA = Occupational Safety and Health Administration; NIOSH = National Institute for Occupational Safety and Health; EU-SCOEL = European Union Scientific Committee on Occupational Exposure Limits
[b] MRL = Minimum Risk Level; TWA = Time Weighted Average; LOAEL = lowest-observed-adverse-effect-level; STEL = Short-term Exposure Limit; NOAEL = no-observed-adverse-effect-level; UF = uncertainty factor; URT = upper respiratory tract; PEL = permissible exposure limit; REL = recommended exposure limit.
[c] One ppm of formaldehye in air is equivalent to 1.23 mg/m3 assuming standard temperature and pressure and based on the MW of 30.03 g/mol and the following equation: mg/m3 = (ppm × MW) / 24.45 L/mol
[d] RfC = POD / UF

Appendix B Benchmark Dose Modeling

B.1 BMD Modeling in Support of Acute Inhalation POD Derivation

The following excerpts are from ICF Memorandum to EPA (2022). Statistical Review of the Andersen and Mølhave and Kulle et al Formaldehyde Inhalation Exposure Studies. September 5, 2022. Please refer to that report for the entire analysis.

Table_Apx B-1 and Table_Apx B-2 present the BMDS model summaries for eye irritation. The results in Table_Apx B-1 are from the IRIS report (EPA, 2022) that used the older BMDS Version 2.2. For comparison, the results in Table_Apx B-2 are from the current BMDS Version 3.3rc10. Note that the IRIS report models do not include the Dichotomous Hill and Multistage Degree 1 models.

Table_Apx B-1. BMDS Version 2.2 Summary for Eye Irritation[a] [b] [c]

Model	BMD (ppm)	BMDL (ppm)	P-value	AIC
Gamma	0.853	0.497	0.182	66.839
Log-Logistic	0.852	0.510	0.147	67.596
Multistage Degree 3	0.863	0.369	0.226	66.134
Multistage Degree 2	0.676	0.395	0.373	65.090
Weibull	0.886	0.501	0.211	66.225
Logistic	0.760	0.546	0.364	64.737
Log-Probit	0.850	0.541	0.159	67.254
Probit	0.694	0.502	0.369	64.645
Quantal Linear	0.270	0.191	0.063	71.876

[a] Results from EPA (2022).
[b] Selected Model Based on Lowest AIC is bolded
[c] Adapted from Table 24a from ICF (2022)

Table_Apx B-2. BMDS Version 3.3rc10 Summary for Eye Irritation

Model	BMD (ppm)	BMDL (ppm)	P-value	AIC
Dichotomous Hill	0.852	0.510	0.415	67.596
Gamma	0.853	0.497	0.437	66.839
Log-Logistic	0.852	0.510	0.415	67.596
Multistage Degree 3	0.863	0.369	0.410	66.134
Multistage Degree 2	0.676	0.395	0.678	65.090
Multistage Degree 1	0.270	0.191	0.280	71.876
Weibull	0.886	0.501	0.395	66.225
Logistic	0.760	0.546	0.608	64.737
Log-Probit	0.850	0.541	0.452	67.254
Probit	0.694	0.502	0.600	64.645
Quantal Linear	0.270	0.191	0.280	71.876

[a] Results from EPA (2022).
[b] Selected Model Based on Lowest AIC is bolded
[c] Adapted from Table 24a from ICF (2022)

For both BMDS versions, the selected model based on the AIC was the Probit model, with the dose response equation: P(response) = CumNorm(a+b*Dose). For both BMDS versions, the (rounded) BMD and BMDL were 0.694 and 0.502 ppm, respectively. The BMD, BMDL, and AIC values for the two BMDS versions were all within 0.001 of each other, strongly suggesting that both versions used the same modeling formulations and data; the slight differences are likely due to differences in the convergence criteria.

The p-values for the two BMDS versions are extremely different. For example, the p-value for the selected model using BMDS Version 2.2 was 0.369 but the p-value for the selected model using BMDS Version 3.3rc10 was 0.600. Although documentation for the p-value calculations used in BMDS Version 2.2 could not be found, the values in BMDS Version 3.3rc10 agree with the usual p-value approach described on page 67 of the *Benchmark Dose Technical Guidance* (EPA, 2012): The scaled residuals for each dose (not shown here) are (O-E)/sqrt(E), where O and E are the observed and expected counts, the chi-squared statistic (1.871) is the sum of the squared scaled residuals, and the p-value (0.600) is indeed the probability that a chi-square value with 3 degrees of freedom exceeds 1.871.

B.2 BMD Modeling in Support of Dermal POD Derivation

Two human skin sensitization studies (Flyvholm et al., 1997; Fischer et al., 1995) were considered for inclusion in the benchmark dose (BMD) analysis using the Benchmark Dose Software (BMDS, version 3.3.2, release date: 3-21-2023). Both studies were taken to the HSRB where they agreed with EPA's conclusions that these studies could be used as part of a WOE for a dermal endpoint/Point of Departure (POD) for sensitization (HSRB, 2023b). BMD analysis was recommended by the HSRB to establish a more representative threshold and as a potential way for combining data across multiple studies. There was some concern raised by the HSRB about the reliability of the data for the Fischer et al. study and the TRUE Test patch results, based primarily on inconsistencies in results reporting. Therefore, EPA evaluated the data using the studies alone and together, but only used the patch test results from Fischer et al. when using the study in the BMD analysis. The data was analyzed using a benchmark response (BMR) of 5, 10, and 20 percent to understand the impact on results since a standard BMR for dermal sensitization is not available. The 10 percent BMR was regarded as an appropriate response level for the

data set based on the number of individuals tested (equates to approximately two individuals testing positive). Due to the lack of individual reporting in the studies, data were analyzed as dichotomous where any positive result was considered a positive sensitization reaction regardless of the severity of response (+, ++, +++). Questionable responses were regarded as a negative response for treatment and control data inclusion.

Utilizing human data in BMD analyses can have uncertainty based on study design and data reported in study as they often lack details of analyses or raw data, particularly for studies from open literature. For these studies, data were lacking on the male and female designations for individual study participants; in Flyvholm et al., the total number of males and females that participated in the study was provided but no additional data. For this reason, data analyzed herein reflects the combination of male and female data. Although male and female data is often separated for BMD analyses, as the test population in the study reflected a sensitized population, using both sexes combined was deemed less impactful to the analyses. In both studies, different patch tests were conducted simultaneously on each individual (e.g., occluded and non-occluded, Finn chamber and TRUE Test, etc.) and the range of concentrations within each test system was simultaneously tested on each individual. This is consistent with how most patch testing is conducted in a clinical setting where multiple allergens are tested at the same time.

While these tests are by design meant to give independent results, there is some uncertainty if cross reactivity could occur from simultaneous testing, as referenced by the HSRB as "excited back syndrome" (Duarte et al., 2002). Although this phenomenon has been reported, it has also been described as not being reproducible in controlled testing even in individuals that had previously reported this syndrome (Andersen et al., 1993). There are potentially more complex models beyond BMDS that could be explored (e.g., multiple outcome models) to help explain potential correlation between the outcomes of simultaneous tests; however, this is beyond the capabilities of the BMD software, and would potentially provide limited additional information useful for setting the BMD. Additionally, the outcome obtained in the BMD analysis would likely be more conservative in nature if there is any increased sensitivity induced by simultaneous testing. Based on these factors, additional testing was not conducted beyond the BMD analysis, although the potential uncertainty in the assumption of independence is recognized.

BMDS version 3.3.2 was used for the analysis, the Microsoft Excel-based version of the tool. A summary table of selected results of the BMD including rationale for curve selection is provided in Table_Apx B-3. The results of all analyses are reflected in the attached workbooks for analyses conducted for each study alone as well as the studies combined. Summary tables and further explanation of curve selection for each of the three analyses are further described below Table_Apx B-3.

Table_Apx B-3. Summary of BMD Analyses for Dermal Skin Sensitization

Study Analysis	BMR = 10%		Model Selected/Rationale
	BMD	BMDL	
Flyvholm	18.2	10.5	Log-probit, best fit, lowest BMDL, AICs relatively close
Fischer (patch only)	10.1	5.9	Log-logistic, lowest AIC, good curve fit
Flyvholm and Fischer (patch only)	12.6	10.6	Multistage Degree 2, lowest AIC
	12.1	8.6	Log-probit, lowest BMDL, similar AIC (shown for comparison to log-probit based on Flyvholm alone)

BMD Analysis of Flyvholm et al., 1997 Occluded Patch Test Results

The detailed summary table for Flyvholm et al, 1997 analysis is shown in Table_Apx B-4 and Table_Apx B-5. Table_Apx B-4 represents results in units presented in the study (ppm) while Table_Apx B-5presents units of μg/cm^2. The results are equivalent if converted before or after the analysis and are therefore just presented in μg/cm^2 for the BMD analyses for Fischer and the combined studies analysis. Based on the criteria of lowest AIC alone, multistage degree and quantal could be considered viable model choices, and yield BMDL values in the range of 12 to 15 μg/cm^2. The log-probit model was also considered as it yielded the lowest BMDL at 10.5 μg/cm^2. Visual curve inspection was performed for all of these models, and the log-probit curve appeared to give the best fit of the data at the low end of the curve. Considering this factor in addition to the relatively close range of AIC values (77–84) and this representing a more conservative BMDL selection, the log-probit model was selected for the BMDL. This is also more consistent with the BMDL from the combined studies and the Fischer study alone analyses.

Table_Apx B-4. Summary of BMD Model Output from of Flyvholm et al., 1997 Occluded Patch Test Results (ppm or mg/L) [a]

Model	Analysis Type	Restriction	RiskType	BMRF	BMD	BMDL	BMDU	P Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Dichotomous Hill	frequentist	Restricted	Extra Risk	0.1	670.874	368.228	1090.990	0.431	83.974	0.537	1.590	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	695.152	437.577	1128.297	0.669	81.769	0.526	1.142	Viable - Alternate	
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	670.874	368.228	1091.462	0.431	83.974	0.537	1.590	Viable - Alternate	
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	841.345	511.311	1670.243	0.953	77.490	0.443	0.361	Viable - Alternate	
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	812.807	473.845	1547.492	0.871	78.593	0.362	0.728	Viable - Alternate	
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	576.351	428.756	791.848	0.775	80.140	0.200	1.348	Viable - Alternate	
Weibull	frequentist	Restricted	Extra Risk	0.1	735.545	442.903	1225.201	0.680	81.562	0.621	1.037	Viable - Alternate	
Logistic	frequentist	Unrestricted	Extra Risk	0.1	2200.154	1690.256	2824.260	0.481	84.454	1.438	-0.027	Viable - Alternate	
Log-Probit	**frequentist**	**Unrestricted**	**Extra Risk**	**0.1**	**604.874**	**349.018**	**931.915**	**0.391**	**84.269**	**0.391**	**1.622**	**Viable - Recommended**	**Lowest BMDL**
Probit	frequentist	Unrestricted	Extra Risk	0.1	1988.859	1550.734	2533.751	0.528	83.979	1.353	-0.041	Viable - Alternate	
Quantal Linear	frequentist	Unrestricted	Extra Risk	0.1	576.351	428.752	791.838	0.775	80.140	0.200	1.348	Viable - Alternate	

[a] Conversion of BMDL based on ppm to% to µg/cm^2: Based on 15 µl solution used and 0.8 cm diameter of Finn test chamber; 349.02 ppm = 0.0349% = 349.02 mg/L and (349.02 mg/L)(1000 µg/1 mg)(15 µL/π(0.4 cm)^2)(1 L/10^6 µL) = 10.47 µg/cm^2

Table_Apx B-5. Summary of BMD Model Output from of Flyvholm et al., 1997 Occluded Patch Test Results (µg/cm^2)

Model	Analysis Type	Restriction	RiskType	BMRF	BMD	BMDL	BMDU	P Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Dichotomous Hill	frequentist	Restricted	Extra Risk	0.1	20.126	11.047	32.730	0.431	83.974	0.537	1.590	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	20.855	13.127	33.849	0.669	81.769	0.526	1.142	Viable - Alternate	
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	20.126	11.047	32.744	0.431	83.974	0.537	1.590	Viable - Alternate	
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	25.240	15.339	50.107	0.953	77.490	0.443	0.361	Viable - Alternate	
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	24.384	14.215	46.425	0.790	80.593	0.362	0.728	Viable - Alternate	
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	17.291	12.863	23.755	0.775	80.140	0.200	1.348	Viable - Alternate	
Weibull	frequentist	Restricted	Extra Risk	0.1	22.066	13.287	36.756	0.680	81.562	0.621	1.037	Viable - Alternate	
Logistic	frequentist	Unrestricted	Extra Risk	0.1	66.005	50.708	84.728	0.481	84.454	1.438	-0.027	Viable - Alternate	
Log-Probit	**frequentist**	**Unrestricted**	**Extra Risk**	**0.1**	**18.146**	**10.471**	**27.957**	**0.391**	**84.269**	**0.391**	**1.622**	**Viable - Recommended**	**Lowest BMDL**
Probit	frequentist	Unrestricted	Extra Risk	0.1	59.666	46.522	76.013	0.528	83.979	1.353	-0.041	Viable - Alternate	
Quantal Linear	frequentist	Unrestricted	Extra Risk	0.1	17.291	12.863	23.755	0.775	80.140	0.200	1.348	Viable - Alternate	

BMD Analysis of Fischer et al., 1995 Patch Test Results Only

The detailed summary table for Fischer et al., 1995 analysis is shown in Table_Apx B-6. The BMDs/BMDLs are fairly consistent across models with comparable AIC values, yielding higher confidence in the results. The logistic model produces very different BMD estimates, based on the curve fit and the AIC poorly approximates the dose-response relationship. The reviewer agreed with the software selected model based on the lowest AIC, lowest BMDL and visual inspection of the curve fit of the data.

Table_Apx B-6. Summary of BMD Model Output from of Fischer et al., 1995 Patch Test Results Only (µg/cm^2)

Model	Analysis Type	Restriction	RiskType	BMRF	BMD	BMDL	BMDU	P Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Dichotomous Hill	frequentist	Restricted	Extra Risk	0.1	10.808	6.122	16.006	0.977	164.075	-0.103	0.066	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	10.446	8.481	14.271	0.615	163.929	-0.212	-1.664	Viable - Alternate	
Log-Logistic	**frequentist**	**Restricted**	**Extra Risk**	**0.1**	**10.106**	**5.936**	**15.162**	**0.984**	**162.336**	**-0.243**	**-0.308**	**Viable - Recommended**	**Lowest AIC**
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	10.446	8.481	13.072	0.615	163.929	-0.212	-1.664	Viable - Alternate	
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	10.446	8.481	13.072	0.615	163.929	-0.212	-1.664	Viable - Alternate	
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	10.446	8.481	13.047	0.615	163.929	-0.212	-1.664	Viable - Alternate	
Weibull	frequentist	Restricted	Extra Risk	0.1	10.446	8.481	13.809	0.615	163.929	-0.212	-1.664	Viable - Alternate	
Logistic	frequentist	Unrestricted	Extra Risk	0.1	31.301	25.289	38.829	<0.0001	188.717	1.233	-3.275	Questionable	Goodness of fit p-value < 0.1
Log-Probit	frequentist	Unrestricted	Extra Risk	0.1	9.987	6.140	14.484	0.974	162.535	-0.254	-0.291	Viable - Alternate	
Probit	frequentist	Unrestricted	Extra Risk	0.1	32.456	26.884	39.353	<0.0001	190.543	1.269	-2.801	Questionable	Goodness of fit p-value < 0.1
Quantal Linear	frequentist	Unrestricted	Extra Risk	0.1	10.446	8.481	13.047	0.615	163.929	-0.212	-1.664	Viable - Alternate	

BMD Analysis of Combined Data from Flyvholm et al. and Fischer et al., Patch Test Results Only

The detailed summary table for the BMD analysis of combined data from Flyvholm et al. and Fischer et al., (patch test results only) is shown in Table_Apx B-7. Although the studies were analyzed as a combined dataset based on the HSRB recommendation, this is not a common practice within EPA OPP; however, guidance for considering combining study data is provided in the Benchmark Dose Software v3.3. User Guidance (see Section 14.3 Test for Combining Two Datasets for the Same Endpoint). This guidance was followed for the BMD analyses presented here using the separate and combined output, which looks at comparing the maximum log-likelihood using the data combined or separately, and then comparing differences to a Chi-squared distribution (following steps in Section 14.3). Following this guidance, the null hypothesis that the two sets have the same dose-response relationship (based on being greater than the 95th percentile of the Chi-square distribution) was *not* rejected, suggesting combining the data sets may be a valid analysis. However, the BMDL results from the combined dataset was only used to explore the impact of combining the data from both studies and the BMDL from the single study was used in POD selection.

Results from the combined data set were similar to the output obtained from the BMD analysis from the individual studies. There are no real differences between the AICs and BMD estimates for several models: gamma, multistage (1, 2, and 3 degree), Weibull and quantal linear. There is another cluster of models that are only slightly worse in fit and the BMD estimates are only slightly different: log-probit, log-logistic and dichotomous Hill. All of these models provide fairly consistent BMDLs between 9 and 11. The reviewer agreed with the software selected Multistage Degree 2 model based on the lowest AIC and visual inspection of the curve fit of the data, although multiple models satisfied these criteria. The log-probit model result was also included in the summary table above for comparison to the Flyvholm et al. log-probit results and as an example of models with lower BMDL values and similar AIC (within <1) to the Multistage Degree 2 model (Dichotomous Hill, log-logistic and log-probit).

Table_Apx B-7. Summary of BMD Model Output from Flyvholm et al. and Fischer et al., Patch Test Results Only

Model	Analysis Type	Restriction	RiskType	BMRF	BMD	BMDL	BMDU	P Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Dichotomous Hill	frequentist	Restricted	Extra Risk	0.1	12.415	8.515	17.022	0.731	247.963	-0.327	-0.245	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	12.549	10.573	16.696	0.702	247.194	-0.254	-0.355	Viable - Alternate	
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	12.415	8.515	17.021	0.731	247.963	-0.327	-0.245	Viable - Alternate	
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	12.549	10.573	15.545	0.702	247.194	-0.254	-0.355	Viable - Alternate	
Multistage Degree 2	**frequentist**	**Restricted**	**Extra Risk**	**0.1**	**12.549**	**10.573**	**15.545**	**0.702**	**247.194**	**-0.254**	**-0.355**	**Viable - Recommended**	**Lowest AIC**
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	12.549	10.573	14.995	0.702	247.194	-0.254	-0.355	Viable - Alternate	
Weibull	frequentist	Restricted	Extra Risk	0.1	12.549	10.573	16.393	0.702	247.194	-0.254	-0.355	Viable - Alternate	
Logistic	frequentist	Unrestricted	Extra Risk	0.1	41.021	34.770	48.345	<0.0001	283.491	2.337	-1.464	Questionable	Goodness of fit p-value < 0.1 | Residual for Dose Group Near BMD| > 2
Log-Probit	frequentist	Unrestricted	Extra Risk	0.1	12.120	8.561	16.187	0.744	247.719	0.139	-0.089	Viable - Alternate	
Probit	frequentist	Unrestricted	Extra Risk	0.1	40.173	34.614	46.660	<0.0001	283.234	2.353	-1.434	Questionable	Goodness of fit p-value < 0.1 | Residual for Dose Group Near BMD| > 2
Quantal Linear	frequentist	Unrestricted	Extra Risk	0.1	12.549	10.573	14.995	0.702	247.194	-0.254	-0.355	Viable - Alternate	

B.3 Appendix B References

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