OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION
WASHINGTON, D.C. 20460

September 5, 2024

MEMORANDUM

SUBJECT: Revised Human Health and Ecological Risk Assessment for the Proposed New Active Ingredient Glycerol Formate as a Non-Food Contact Disinfectant

PC Code: 087803	DP Barcode: 455166
Decision No.: 556590	Registration No(s).: 1677-EAA
Regulatory Action: PRIA Risk Assessment	CAS No.: 82905-59-7

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This document provides the human health and ecological risk assessment conducted in support of the proposed new active ingredient (a.i.) registration of glycerol formate as a disinfectant for use on hard, non-porous, non-food contact surfaces and as a sanitizer for use on soft, surfaces in healthcare settings such as hospitals, medical premises/equipment, and nursing homes.

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1 EXECUTIVE SUMMARY

Ecolab, Inc. has submitted a request for one end-use product (DuoGuard RTU, EPA Reg. No. 1677-EAA) containing the proposed new active ingredient, glycerol formate (PC Code 087803). Glycerol formate is a blend of three ester forms: glycerol monoformate, glycerol diformate, and glycerol triformate. The end-use product is intended to be used as a disinfectant for non-porous, non-food contact hard surfaces and as a sanitizer for soft surfaces in healthcare settings (e.g., hospitals, medical premises/equipment, and nursing homes). Based on the label, two parts (Part A containing 99.7% glycerol formate and Part B containing 1.1% hydrogen peroxide) are mixed to generate performic acid (PFA; also known as peroxyformic acid, CAS No. 107-32-4) *in situ*. Primary exposure will be to PFA as the active biocide, which, according to the label (dated May 9, 2022), lasts for approximately 1 hour from the time the ingredients are combined. The Agency does not have human health toxicological data for PFA. Therefore, the Agency is relying on the toxicity database for hydrogen peroxide based on the established partial read-across for PFA, peroxyacetic acid, and hydrogen peroxide.

Human Health Risk Summary

The anticipated exposure pathway of concern from use of the DuoGuard RTU product is inhalation exposure to PFA. While the Agency does not have toxicity data specific to PFA, through the Agency's analysis and information submitted by the registrant, the Agency agreed that PFA may generally be grouped with the peroxy compounds (Appendix C). However, given the greater reactivity of PFA, and, thus, the greater potential for toxicity and the limited toxicological database for hydrogen peroxide, a database uncertainty factor of 10x is applied to the Level of Concern (LOC) for the Margin of Exposure (MOE). Based on this information, inhalation exposures and risks were assessed using the No Observed Adverse Effect Concentration (NOAEC) of 10 mg/m^3 as the point of departure, which was the highest dose tested in a 90-day rat inhalation toxicity study (MRID 49469301) with hydrogen peroxide. This Point of Departure (POD) is supported by results of a 28-day inhalation toxicity study (CEFIC Peroxygen Sector Group, 2002) in rats with hydrogen peroxide, where necrosis and inflammation of squamous epithelium and anterior nasal cavity were observed at the Lowest Observed Adverse Effect Concentration (LOAEC) of 14.6 mg/m^3. The NOAEC of 10 mg/m^3 was converted to an 8-hour Human Equivalent Concentration (HEC) of 7.5 mg/m^3 (5.4 ppm) and a 24-hour HEC of 2.5 mg/m^3 (1.8 ppm) based upon the rat exposure time of 6 hours per day and a regional gas deposition ratio (RGDR) of 1.0. A total uncertainty factor (UF) of 100x was used which includes the UF_A of 3x for interspecies variation, the UF_H of 3x for intraspecies variation and the UF_{DB} of 10x for the uncertainties from utilizing hydrogen peroxide toxicity data to assess the toxicity of PFA.

The occupational handler inhalation MOE for PFA was calculated using the PFA sample data and predicted PFA concentrations from the submitted exposure study (MRID 51245501) and titration study (MRID 52062301), respectively. The MOE is 110 and not of concern because it is greater than the LOC of 100.

The residential bystander inhalation MOE for PFA was calculated using the PFA sample data for the first hour of exposure and one half the LOD of 0.003 ppm for the remaining 23 hours of exposure. The MOE is 600 and not of concern because it is greater than the LOC of 100.

Ecological Risk Summary

Due to the reaction of glycerol formate with hydrogen peroxide to form PFA (the final active biocide), primary exposure is expected to be to PFA. Based on the indoor use pattern, high volatility, and rapid degradation in the environment, environmental exposure to PFA is expected to be minimal from the antimicrobial uses of glycerol formate. The Agency does not anticipate risks to terrestrial and aquatic non-target organisms from the proposed antimicrobial uses of glycerol formate and does not have a reasonable expectation of discernible direct or indirect effects to threatened or endangered (listed) species or their designated critical habitat from these glycerol formate uses. Therefore, the Agency has made a “no effect” determination for glycerol formate under the Endangered Species Act (ESA) for all listed species and designated critical habitats for such species.

2 INTRODUCTION

The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

2.1 Chemical Overview

The risk assessment conducted for this action under the Pesticide Registration Improvement Act (PRIA) is for the proposed new active ingredient (a.i.) glycerol formate (PC Code 087803, CAS No. 82905-59-7). Glycerol formate is a blend of three ester forms: glycerol monoformate, glycerol diformate, and glycerol triformate. The proposed end-use product (DuoGuard RTU, EPA Reg. No. 1677-EAA) is a two-part system with Part A containing 99.7% glycerol formate and Part B containing 1.1.% hydrogen peroxide. Per the label, Part A and Part B are mixed to generate performic acid (PFA; also known as peroxyformic acid, CAS No. 107-32-4) *in situ*. Primary exposures from the proposed use are to PFA. Hydrogen peroxide was previously registered for the proposed use pattern and assessed within the Registration Review for Antimicrobial Uses of the Peroxy Compounds (Cases 6059, 4072 and 5081; US EPA, 2022). It should be noted that there are two formic acid products (PC Code 214900, CAS No. 64-18-6; EPA Reg. Nos. 90924-13 and 1677-269) used in closed loading and delivery systems in oil-field production that, when mixed with hydrogen peroxide, also generate PFA *in situ*.

2.2 Ingredient Profile and Chemical Identity

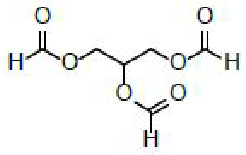
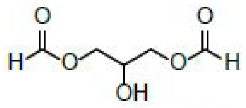
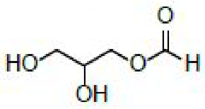
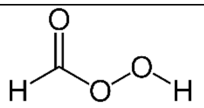
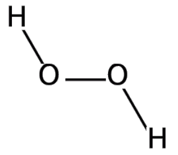
Glycerol formate is formed by the esterification reaction of glycerol with formic acid and is a blend of three ester forms: glycerol monoformate, glycerol diformate, and glycerol triformate (MRID 50864501). The triester form (glycerol triformate) is the predominant form in glycerol formate and drives the rapid production of PFA (MRIDs 50864504 and 51805101). Glycerol formate reacts with hydrogen peroxide to form PFA, the active biocide, as illustrated in the reactions below (MRID 50864504):

Glycerol triformate + $H_2O_2 \rightarrow$ performic acid + glycerol diformate

Glycerol diformate + H_2O_2 → performic acid + glycerol monoformate
Glycerol monoformate + H_2O_2 → performic acid + glycerol

In an aqueous environment, glycerol formate will rapidly degrade to glycerol and formate/formic acid. Similarly, PFA, a highly unstable strong oxidant, will degrade rapidly to formate/formic acid and water. Table 1 below lists the chemical identity and physical properties of glycerol formate and PFA, the active biocide.

Table 1. Physical-Chemical Properties of Glycerol Formate, PFA, and Hydrogen Peroxide

Property	Glycerol Formate	Performic Acid (Peroxyformic Acid; PFA)	Hydrogen Peroxide
Chemical Classification	Ester	Percarboxylic acid (or peracid)	Peroxide
PC Code	087803	N/A (not registered as an a.i.)	000595
CAS No.	82905-59-7	107-32-4	7722-84-1
SMILES Code	C(C(COC=O)OC=O)OC=O (glycerol triformate)	C(=O)OO	OO
Molecular Formula	$C_6H_8O_6$ (glycerol triformate)	CH_2O_3	H_2O_2
Molecular Weight (g/mol)	176.124 (glycerol triformate)	62.025	34.01
Molecular Structure	Glycerol triformate  Glycerol diformate  Glycerol monoformate 		
pH @ 25°C	3.86 (1% w/w solution), 4.14 (neat) (MRID 50864502)[1]	No data	4.3 (based on 50% solution; NCBI, 2021a)
UV/Visible Absorption	Maximum absorption at 206-218 (<290-800 nm)[1] (MRID 50864502). Not expected to photolyze.	No data	No light absorption above 290 nm (Molina *et al.*, 1977)
Melting Point (°C)	N/A (liquid) (MRID 50864502)	N/A (liquid)	-0.43 (EPI-Suite, v4.11, exp)
Boiling Point (°C)	237.3 (MRID 50864502)[1]	127.5 (EPI-Suite, v4.11, exp)	152 (EPI-Suite, v4.11, exp)
Density (g/mL)	1.313 (MRID 50864502)[1]	1.341 (ChemSrc, 2021)	1.44 (NCBI, 2021a)

Property	Glycerol Formate	Performic Acid (Peroxyformic Acid; PFA)	Hydrogen Peroxide
Octanol-Water Partition Coefficient @ 25°C (log K_{ow})	-0.84 (MRID 50864502)[1]	-1.62 (EPI-Suite, v4.11, est)	-1.57 (EPI-Suite, v4.11, est)
Water Solubility (mg/L) @ 25°C	>5.8 x 10^5 @ 24°C (MRID 50864502)[1]	1 x 10^6 (EPI-Suite, v4.11, exp)	1 x 10^6 (EPI-Suite, v4.11, exp)
Dissociation Constant (pK_a)	No data	7.1 (Rappoport, 2006, p. 698) 7.77 (NCBI, 2021c)	11.62 (NCBI, 2021a)
Vapor Pressure (mmHg) @ 25°C	0.219 @ 20°C (MRID 50864502)[1]	77.7 (EPI-Suite, v4.11, est)	1.97 (EPI-Suite, v4.11, exp)
Henry's Law Constant (atm·m^3/mol) @ 25°C	<10^{-8} (glycerol triformate) (EPI-Suite, v4.11, est)	<10^{-5} (EPI-Suite, v4.11, est)	7.04 x 10^{-9} (EPI-Suite, v4.11, exp)

NA = Not Applicable; exp = experimental data; est = estimated.

[1] For MUP product 919789 (EPA Reg. No. 1677-EAL, 99.83% a.i. purity), which is comprised of 45-60% glycerol triformate, 36-48% diformate, and 0-10% monoformate (based on MRID 50864501).

2.3 Use Pattern

The proposed glycerol formate product is labeled for use as a disinfectant for use on non-porous, non-food contact hard surfaces and as a sanitizer for soft surfaces in healthcare settings. Use sites include hospitals, medical premises/equipment, and nursing homes/assisted living facilities. Hard surfaces include non-critical and semi-critical stainless-steel instruments, toilet bowls, walls, bathrooms, etc., while soft surfaces include cotton or polyester sheets, towels, and upholstery.

As described previously, the proposed end-use product (DuoGuard RTU, EPA Reg. No. 1677-EAA) is a two-part system with Part A containing 99.7% glycerol formate and Part B containing 1.1% hydrogen peroxide. Per the label, Part A and Part B are mixed to generate PFA *in situ*. PFA, by itself, is highly unstable and will degrade rapidly; however, the proposed product contains inert ingredients to stabilize PFA. In addition, the higher-level (tri and di) ester forms of glycerol formate break down into lower-level ester forms (di and mono) in a cascading fashion, continuously feeding into the production of PFA during the product use life (MRID 50864504). The use solution containing the PFA is then applied to hard, non-porous surfaces or soft surfaces using a cloth wipe for the purpose of disinfecting or sanitizing those surfaces. Primary exposure will be to PFA, which, according to the label (dated May 9, 2022), lasts for approximately 1 hour from the time the ingredients are combined.

The combined use solution contains an initial concentration of 800 ppm of PFA (maximum concentration at 5 minutes), with a target PFA concentration of at least 300 ppm throughout the use life (up to 60 minutes; minimum of 300 ppm at 60 minutes) (MRIDs 50864504 and 52062301). The solution is efficacious for 60 minutes after mixing and a new batch will need to be mixed every 60 minutes.

The directions for use indicate that:

- To disinfect "Allow surfaces to remain wet for [appropriate] [listed] [stated] contact time. [No rinsing necessary.] Allow to air dry, or if desired, remove solution with a clean wet cloth."
- To sanitize "Allow to remain wet for [appropriate] [listed] [stated] contact time. Allow to air dry."

The contact times range from 90 seconds for soft surface sanitizing to four minutes for bacterial biofilm control. The contact times are one to three minutes for viruses and three minutes for bacteria, spores, and yeasts.

3 HUMAN HEALTH RISK ASSESSMENT

3.1 Data Deficiencies

As stated previously, primary exposure is to PFA, formed by the reaction of glycerol formate and hydrogen peroxide.

In lieu of providing the toxicity data required for the proposed registration of a new active ingredient as outlined in 40 CFR §158.2230, the registrant provided a rationale for bridging PFA to the peroxy compounds for the purpose of conducting the risk assessment (MRID 51476901). EPA considered the rationale submitted by the registrant as well as additional information as outlined in the OECD Guidance on Grouping of Chemicals (OECD, 2014). Using this information, the Agency assembled a partial read-across for PFA, peracetic acid (also known as peroxyacetic acid; PAA), and hydrogen peroxide and concludes that PFA may generally be grouped with the peroxy compounds (Appendix C). However, given the greater reactivity of PFA, and, thus, the greater potential for toxicity and the limited toxicological database for hydrogen peroxide, a database uncertainty factor (UF_{DB}) of 10x is applied to the Level of Concern (LOC) for the Margin of Exposure (MOE) (Appendix C). The LOC is 100x for all inhalation exposure durations. This LOC includes the UF_{DB} of 10x, the UF_A of 3x for interspecies variation, the UF_H of 3x for intraspecies variation.

The toxicology database for the peroxy compounds (hydrogen peroxide, peracetic acid, potassium peroxymonosulfate, potassium peroxymonosulfate sulfate, peroxyoctanoic acid, and sodium percarbonate) is considered adequate for this risk assessment.

3.2 Label Recommendations

The section of the label (dated May 9, 2022) that begins “To clean and deodorize toilets” needs to be deleted. The section beginning “To disinfect [non-critical] [or] [Semi-Critical] Pre-Cleaned Instruments” needs to be deleted. In both cases, only wipe uses were assessed; however, these non-wipe uses involve squirting liquid (1/2 cup or more) and bathing instruments in the solution. The submitted data support the wipe uses only; therefore, a safety finding could not be made for these non-wipe uses and therefore, they need to be deleted from the label.

3.3 Anticipated Exposure Pathways

The anticipated exposure pathway (occupational handler and residential bystander) assessed in this risk assessment is inhalation of PFA formed from the proposed use of glycerol formate in combination with hydrogen peroxide. Exposures to glycerol formate and hydrogen peroxide are not anticipated because of the way the proposed product is packaged and applied. The proposed product is a two-part system where Part A (containing glycerol formate) is contained in a sealed bottle cap and Part B (containing hydrogen peroxide) is contained in a sealed bottle. To create the end use solution, the label directions state: “twist the dosing cap on the bottle to align notch, then press down to break inner seal so that the soultion in the cap fully releases into the bottle. Shake vigorously for 30 seconds to fully mix solution” (label dated May 9, 2022). The end use solution containing PFA is then poured into a container and applied with a wipe to disinfect surfaces. Although dermal exposures can occur from the proposed hospital, medical premises/equipment, and nursing homes/assisted living facilities uses, the percentage of active ingredient (in the end use solution) is low enough that dermal acute toxicity is not expected. It should be noted that this risk assessment applies only to the currently proposed product at the specific rates that have been assessed here and would need to be re-assessed if the physical form of the active ingredient, the application rate, or the proposed uses change. There are no endpoints selected for oral exposure. Dietary exposure is not anticipated from the proposed use in hospital settings.

3.4 Hazard Characterization and Dose-Response Assessment

3.4.1 Toxicology Studies Available for Analysis

As stated above, the Agency does not have mammalian toxicology data for or PFA. Therefore, the Agency is relying on the toxicology database for hydrogen peroxide based on the established partial read-across for PFA, PAA, and hydrogen peroxide (Appendix C). The peroxy compounds database is considered adequate for hazard characterization and toxicity endpoint selection for hydrogen peroxide and contains the following acceptable studies:

- Developmental toxicity study in mice (MRID 46833610)
- The effects of chronic peroxide intake on the peroxide metabolism enzyme activities of rat organs (MRID 46833618)
- 28-day inhalation toxicity study in rats (CEFIC Peroxygen Sector Group, 2002)
- 90-day inhalation study in rats (MRID 49469301)

The toxicity studies were reviewed for the purposes of characterizing hazard and establishing points of departure (PODs) and endpoints to inform the risk from exposures to PFA.

3.4.2 Summary of Toxicological Effects

The US Food and Drug Administration (FDA) has designated hydrogen peroxide as Generally Recognized as Safe (GRAS; 21 CFR §184.1366). Hydrogen peroxide is also found in the human body and can play an important role in cellular defense. The two main cellular enzymes involved in breakdown of hydrogen peroxide are catalase and glutathione peroxidase. Hydrogen peroxide rapidly dissociates into oxygen and water, thus mitigating concern for oral exposures.

Inhalation is the primary exposure route of concern, as the dissociation process produces reactive oxygen species (ROS), which are highly reactive and may cause oxidative damage to biological systems (this is the pesticidal mode of action). High concentrations of peroxy compounds are corrosive and can be acutely toxic and/or extremely irritating to the lungs and skin. Hydrogen peroxide is volatile, with a vapor pressure of ~1-5 mmHg (value highly dependent on concentration and temperature). The average half-life of hydrogen peroxide in air is about 1 day (US EPA, 2007).

In a non-guideline developmental toxicity study (MRID 46833610), a mixture of 40% peracetic acid, 27% acetic acid, 14% hydrogen peroxide, and stabilizers was administered to presumed pregnant ICR mice via whole-body inhalation exposure at concentrations of 0 (distilled water), 1%, or 5% commercial formulation (equivalent to 0, 20, or 100 mg/m^3 peracetic acid vapor) twice daily for 10 min/exposure from post-conception until the 19th day of pregnancy. At 100 mg/m^3 peracetic acid, significant ($p<0.10$) decreases in fetal body weight (13-18%) and fetal body length (6-8%) were observed compared to controls. At 20 mg/m^3 peracetic acid, fetal body weights and length were comparable to controls. The developmental LOAEC is 100 mg/m^3 peracetic acid based on decreased fetal body weight and body length. The developmental NOAEC is 20 mg/m^3.

A non-guideline study (MRID 46833618) was performed to establish whether the levels of peroxide metabolizing enzymes in rat tissues were affected by repeated daily oral intake of hydrogen peroxide. One-month old CFY inbred rats were exposed to 0.5% hydrogen peroxide in their drinking water for two months; rats given untreated water served as controls. At three months of age, the rats were killed and tissues and hemolysate were collected. The activities of superoxide dismutase, peroxidase, and catalase were determined in homogenates of the tissues. Young rats exposed to 0.5% aqueous hydrogen peroxide in drinking water for two months displayed increased activity of the peroxide metabolizing enzymes in various tissues.

There are two inhalation studies available for hydrogen peroxide: a 28-day rat study and a 90-day rat study discussed below.

1. 28-day inhalation toxicity study in rats (CEFIC Peroxygen Sector Group 2002):

This study was conducted by the CEFIC Peroxygen Sector Group and was cited in the 2003 European Union Risk Assessment Report on Hydrogen Peroxide (EU, 2003). This study was not submitted to the Agency, and therefore, the Agency does not have access to the complete study report; only the summary of the study included in the EU risk assessment report for hydrogen peroxide is available for review (see US EPA, 2019).

In this study, groups of five male and female Alpk:AprSD (Wistar-derived) rats were exposed (whole-body for 6 hours per day) to 0 (control), 2.9, 14.6, or 33 mg/m^3 hydrogen peroxide vapor for 5 days per week, for a period of 28 days. Clinical signs demonstrated respiratory tract irritation at the exposure levels of 14.6 and 33 mg/m^3, but not at 2.9 mg/m^3. Necrosis and inflammation of the epithelium in the anterior regions of the nasal cavity were found at the two higher concentration levels. In the larynx, mononuclear cell infiltration was seen in two females at the highest exposure concentration. Moreover, one male rat in each exposure group and two female rats in the high concentration group exhibited perivascular neutrophil infiltration in the lungs, and hemorrhage was found in some animals at the two lower concentration levels.

2. 90-day inhalation toxicity study in rats (MRID 49469301):

Hydrogen peroxide was administered by the inhalation route (nose only) to 10 rats/sex/group at 0, 1.43, 3.49 or 9.95 mg/m^3 six hours per day, five days per week. Rats were sacrificed under pentobarbital anesthesia followed by exsanguination from the abdominal aorta. The NOAEC from the 90-day study is 9.95 mg/m^3 (LOAEC not established in this study).

Use of the 28-day and 90-day Studies for Selecting Inhalation Endpoints and PODs.

For inhalation risk assessment, the Agency concluded that both the 28-day rat inhalation study (CEFIC Peroxygen Sector Group, 2002) and 90-day rat inhalation study (MRID 49469301) are considered as co-critical studies in selecting the endpoints and point of departure for inhalation risk assessment. The Agency concluded the effects in the 28-day inhalation study should not be excluded because in the study, in addition to hemorrhage, lung inflammation was noted in at least one animal in each treated group, along with other effects in the nasal cavity and larynx.

Mutagenicity and Carcinogenicity of PFA

The Agency does not have toxicological data for PFA to fulfill the mutagenicity and carcinogenicity data requirements. Therefore, the Agency relied on the toxicity database for hydrogen peroxide based on the established partial read-across for PFA, peroxyacetic acid, and hydrogen peroxide (See Appendices A and C). With oral exposure, PFA breaks down rapidly in the body; therefore, the Agency does not have a concern for mutagenicity or carcinogenicity via the oral route of exposure. However, the Agency cannot make the same determination for exposure via the inhalation route, as PFA (along with the other peroxy compounds) is a member of the reactive oxygen species family, which are irritants in the lung respiratory tract that can cause oxidative stress damage to a number of biological systems resulting in everything from inflammation, cardiovascular disease, and ischemia to DNA damage and cancer. This risk assessment addresses irritation which is a precursor to the listed effects and considered as protective.

3.5 Safety Factor for Infants and Children (FQPA Safety Factor)

As glycerol formate and PFA have no proposed food uses and all proposed uses are indoors with a low likelihood of environmental exposure in drinking water to glycerol formate and PFA, there are no potential dietary exposures from the proposed uses and therefore the Food Quality Protection Act (FQPA) does not apply.

3.6 Toxicity Endpoint and Point of Departure Selections

POD and endpoint selection for oral exposures (any duration, dietary or incidental) are not needed as there are no oral exposures based on the use pattern for glycerol formate. Although dermal exposures can occur, for this use pattern the percentage of active ingredient (in the end use product) is low enough that dermal acute toxicity is not expected. Therefore, no dermal points of departures are established in this risk assessment.

Acute toxicity data are not available for technical grade PFA. Available acute toxicity information indicates that peracetic acid is of low oral (Toxicity Category III, see Appendix A) and moderate dermal and inhalation toxicity (Toxicity Category II). However, peracetic acid is corrosive and a severe dermal and eye irritant (Toxicity Category I for both).

For the inhalation risk assessment for all peroxy compounds, the Agency concluded that both the 28-day rat inhalation study (CEFIC Peroxygen Sector Group, 2002) and 90-day rat inhalation study (MRID 49469301) are co-critical studies to use as endpoints and PODs. The available data indicated that the effect at the LOAEC of 14.6 mg/m^3 is indicative of an irritation effect (necrosis and inflammation of the epithelium in the anterior regions of the nasal cavity). Effects indicative of irritation are more dependent upon the exposure concentration than total exposure over time or body burden. Thus, Haber's rule (i.e., $C \times t = k$, where C = exposure concentration, t = exposure duration, and k = a constant) is not always applicable in the case of chemicals whose primary toxic effect is irritation.

For hydrogen peroxide, which is highly water-soluble, the effects of concern are limited to the extra-thoracic region including necrosis and inflammation of squamous epithelium in the anterior nasal cavity. No other effects were noted. In accordance with the EPA guidance document "Advances in Inhalation Gas Dosimetry for Derivation of a Reference Concentration (RfC) and Use in Risk Assessment" (US EPA, 2012a), the Agency concluded that the peroxy compounds should be classified as Category 1 gas (highly water-soluble, highly reactive). In calculating the human equivalent concentration (HEC), the regional gas deposition ratio (RGDR) should be set as 1.

As noted in the 28-day inhalation study, the effect of necrosis and inflammation of the epithelium in the anterior regions of the nasal cavity is considered an irritant effect. Compared to systemic adverse effects, irritation and/or corrosion responses are not expected to show as large a variation in severity and duration of response between or among mammalian species. In addition, direct irritation and/or corrosivity effects are more dependent on the concentration at the site(s) of contact compared to the duration of contact, in contrast to systemic toxicity, which is driven by the overall body burden of the chemical. Thus, irritant and/or corrosion effects do not typically increase in severity with continued exposure, and variation among species or within species is not expected to show as large a variation in response as can be observed with systemic effects. Based on recommendations published by the National Research Council (NRC, 2001), for direct-acting irritants and corrosives that do not show systemic toxicity as the primary toxic effect, the UF_A and UF_H can be reduced from 10x to 3x for a total UF of 10x. Increased sensitivity to children was not observed in the toxicity database and the POD based on irritant effects is protective of systemic effects.

For PFA, utilizing the HEC, a total Uncertainty Factor (UF) of 100x is applied for all inhalation exposure durations. This UF includes the UF_A of 3x for interspecies variation and the UFH of 3x for intraspecies variation as discussed above. In addition, a UF_{DB} of 10x is included to account for the uncertainties from utilizing hydrogen peroxide toxicity data to assess the toxicity of PFA.

Table 2. Toxicological Doses and Endpoints for PFA

Exposure Scenario	POD for Risk Assessment	Target MOE[D]	Studies and Observed Effects (Both studies are co-critical)
Performic Acid (Inhalation, All durations[A])	NOAEC = 10 mg/m^3 (7.1 ppm) 8 Hour HEC[B] = 7.5 mg/m^3 (5.4 ppm[C]) 24 Hour HEC[B] = 2.5 mg/m^3 (1.8 ppm[C])	UF_A = 3 UF_H = 3 UF_{DB} = 10 LOC = 100 (Target MOE)	28-day hydrogen peroxide rat inhalation study (CEFIC Peroxygen Sector Group, 2002) 90-day hydrogen peroxide rat inhalation study (MRID 49469301) NOAEC = 10 mg/m^3 (highest dose tested from the 90-day study). LOAEC = 14.6 mg/m^3 (mid-dose from the 28-day study) based on the necrosis and inflammation of squamous epithelium and anterior of the nasal cavity (3/5 M and 2/5 F).

Point of Departure (POD) = A data point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEC = no observed adverse effect concentration. LOAEC = lowest observed adverse effect concentration. HEC = human equivalent concentration. 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_{DB} = use of hydrogen peroxide studies to assess toxicity of PAA and PFA. MOE = margin of exposure. LOC = level of concern. RGDR = regional gas dose ratio.

A. Includes Short Term (1 to 30 days), Intermediate Term (30 to 180 days) and Long Term (more than 180 days)
B. HEC = NOAEC (mg/m^3) * Animal Exposure * (6 hrs/day) / Human Exposure * (8 or 24 hrs/day) * RGDR (1.0)
C. 1 ppm = 1.4 mg/m^3 based on the hydrogen peroxide molecular weight of 34.01 grams/mol.
D. These target MOEs apply to both occupational and residential exposures.

3.7 Dietary Exposure and Risk Assessment

There are no anticipated dietary exposures from the proposed end-use product uses.

3.8 Aggregate Exposure/Risk Characterization

There are no anticipated dietary exposures from the proposed product, thus there is no need for an aggregate exposure characterization.

3.9 Cumulative Exposure/Risk Characterization

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to glycerol formate and any other substances, and glycerol formate does not appear to produce a toxic metabolite produced by other substances. For the purposes of this action, therefore, EPA has not assumed that glycerol formate has a common mechanism of toxicity with other substances. In 2016, EPA's Office of Pesticide Programs released a guidance document entitled, *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis* [https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework]. This document provides guidance on how to screen groups of pesticides for cumulative evaluation using a two-step approach beginning with the evaluation of available toxicological information and if necessary, followed by a risk-based screening approach. This framework supplements the existing guidance documents for establishing common mechanism groups (CMGs)[1] and conducting cumulative risk assessments (CRA)[2].

Glycerol formate and PFA have not been classified in a group for screening. At this time, EPA does not expect any exposures from other pesticides or substances that would warrant screening with the framework. As a result, EPA concludes that glycerol formate and PFA do not have a common mechanism of toxicity with other substances that contribute to the risk assessment. If other pesticides are registered that have the potential to be screened with glycerol formate and PFA, EPA will use the framework to examine the potential for a common mechanism of toxicity and the potential for cumulative risk as part of the ongoing registration review process.

3.10 Occupational Exposure/Risk Characterization

There is the potential for occupational handler inhalation exposure to PFA during the application of the proposed product. The proposed end-use product (DuoGuard RTU, EPA Reg. No. 1677-EAA) is a two-part system with Part A containing 99.7% glycerol formate and Part B containing 1.1% hydrogen peroxide. Per the label, Part A and Part B are mixed to generate PFA

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[1] *Guidance For Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity* (EPA, 1999)

[2] *Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (EPA, 2002)

in situ. These exposures can be long-term in duration because they occur in facilities, such as hospitals, where surface treatments are conducted on a daily basis.

Available Exposure Data

An exposure study (MRID 51245501) was submitted in support of this product. The study occurred in a 2,964 ft^3 six-person bathroom that was meant to simulate the use of the proposed product to disinfect a hospital room and associated single person bathroom. The study report stated that an average hospital room is 320 ft^2, exclusive of bathrooms. With an 8 ft ceiling, that yields a volume of 2,560 ft^3 (72.5 m^3). The bathroom adds an additional 250 ft^3, bringing the total size to approximately 2,800 ft^3 (79.3 m^3).

In this bathroom, the standard airflow rates varied between 150 – 450 cubic feet per minute (CFM). There were two stages to the experiment. In the first, airflow was fixed at a rate that yielded >6 air exchanges per hour (ACH), a target rate for hospital patient rooms. In the second experimental stage, the airflow was set to the lowest possible setting without being turned off completely, equivalent to 1 ACH. Air flow was measured with an air flow capture hood before and after each two-hour block of air sampling, and each reported value was measured at least in triplicate. The supply air entered the bathroom through a diffuser in the ceiling, just to the left of the center of the room. The report does not state how the air exited the room; however, exhaust grills above one or more of the three toilet stalls are visible in the study report photographs.

According to the report, during a hospital room daily clean, typically two microfiber cloths would be used: one for the bathroom and one for the main room. In the bathroom, typically the sink, mirror, toilet, handrails, etc. would be cleaned. In the patient room, high touch surfaces including bed rails, door handles, tray table, call buttons, etc. would be cleaned. Each cloth is wetted with approximately 150 mL of cleaning solution. The same general layout was followed during the bathroom cleaning. Two cloths were wetted with exactly 300 mL of solution. The far stall (largest) was used to approximate the bathroom. One cloth was used to clean the toilet and a section of the divider wall equivalent to the sink and mirror area. The second cloth was used to clean the area surrounding the five sinks. In an actual hospital room, the high touch objects are dispersed throughout the room, and so too is the chemistry application. In this bathroom, the sinks, and therefore the chemistry application, were all centrally located at a single point. Between both locations, the total area cleaned was ~60 ft^2.

Two air sampling pumps (one each for PFA and hydrogen peroxide) were centrally located in the bathroom and left in a fixed location for the duration of the experiment. The cloths were brought into the room, the air sampling pumps were started, and cleaning commenced. After cleaning was concluded, the cloths were removed from the room. PFA was sampled using 15 mL of capture solution in a glass midget impinger. Air was pulled through that solution at a rate of 1 liter per minute (lpm) using a personal air sampling pump. Each sampling interval was 15 min. At the conclusion of that period, 5 mL of solution was transferred from the impinger to a vial, followed by immediate addition of 1-2 mL of thiosulfate to quench any further reaction. Hydrogen peroxide was generally sampled in accordance with the Occupational Safety and Health Administration (OSHA) Method 1019 (OSHA, 2016) using two 25 mm quartz filters coated with titanium oxysulfate hydrate and preloaded into 2-piece polystyrene cassettes (SKC 225-9030). The pumps were operated at 1.5 lpm, however, which is less than the rate of 2.0 lpm in the method.

The PFA air samples were analyzed based on a method that uses the oxidation of methyl p-tolylsulfide (MTS) to methyl p-tolylsulfoxide (MTSO) and is selective for peracids (e.g., peracetic acid or PFA) in the presence of hydrogen peroxide. The report does not provide any information on how this method was validated specifically for PFA; however, it appears to be the same method that was developed and validated by the registrant for PAA (Nordling, 2017). The hydrogen peroxide air samples were analyzed in accordance with the OSHA 1019 method. This method has been validated with a detection limit for the overall procedure (DLOP) of 3.6 µg and the reliable quantitation limit (RQL) of 12.2 µg. Given the sampling volume of 22.5 liters (1.5 lpm for 15 minutes) and using the conversion rate of 1.39 mg/m^3 per ppm, the DLOP is 0.12 ppm and the RQL is 0.39 ppm.

PFA air samples were collected at 15-minute intervals for two hours. The PFA air concentrations were above the limit of detection (LOD) of 0.003 ppm for the first three sampling intervals at 6.8 ACH and they were above the LOD for all four intervals at 1.1 ACH. The samples were below the LOD for the remaining intervals. A summary of the PFA air concentrations for the first hour of sampling is included in Table 3.

Table 3. Performic Acid Concentrations Measured in MRID 51245501

	Ventilation Rate = 6.8 ACH[A]				Ventilation Rate = 1.1 ACH			Overall Average
Time (minutes)	Run 1 (ppm)	Run 2 (ppm)	Run 3 (ppm)	Average (ppm)	Run 1 (ppm)	Run 2 (ppm)	Average (ppm)	
0 to 15	0.083	0.096	0.095	0.091	0.071	0.078	0.075	0.083
15 to 30	0.035	0.049	0.041	0.042	0.028	0.036	0.032	0.037
30 to 45	0.017	0.031	0.021	0.023	0.025	0.017	0.017	0.020
45 to 60	0.005	0.005	<LOD[B]	0.004[C]	0.006	0.005	0.005	0.004

A. ACH = Air Changes per Hour

B. LOD = 0.003 ppm

C. The average was calculated using the LOD of 0.003 ppm

A summary of the hydrogen peroxide air concentrations for the first hour of sampling is included in Table 4. The hydrogen peroxide air concentrations were above the RQL of 0.39 ppm during the first sampling interval. During the second sampling interval, two samples were above the RQL, and the remaining three samples were between the DLOP and the RQL. The samples were below the DLOP for the remaining intervals.

Table 4. Hydrogen Peroxide Acid Concentrations Measured in MRID 51245501

Time (minutes)	Ventilation Rate = 6.8 ACH				Ventilation Rate = 1.1 ACH			Overall Average (ppm)
	Run 1 (ppm)	Run 2 (ppm)	Run 3 (ppm)	Average (ppm)	Run 1 (ppm)	Run 2 (ppm)	Average (ppm)	
0 to 15	0.83	0.77	0.81	0.80	0.64	0.52	0.58	0.69
15 to 30	0.40	0.50	0.31*	0.40	0.29*	0.22*	0.26*	0.33
30 to 45	<DLOP	0.20*	<DLOP	<DLOP	<DLOP	<DLOP	<DLOP	<DLOP
45 to 60	<DLOP	<DLOP	<DLOP	<DLOP	<DLOP	<DLOP	<DLOP	<DLOP

ACH = Air Changes per Hour

DLOP = 0.12 ppm, RQL = 0.39 ppm

*Value is above DLOP but less than RQL

Titration Data of PFA in Solution

Titration data of PFA concentrations in solution (MRID 52062301) were submitted by the registrant in conjunction with the efficacy studies to support the use life of 60 minutes. These data indicate that the PFA concentration rapidly increases for about five minutes after mixing part A and B and then slowly declines. The PFA concentrations were predicted using the following equations:

- For the time period 0 to 5 minutes, $y = 219.4\ln(x) + 506.5$, $R^2 = 0.919$
- For the time period 5 to 65 minutes, $y = 880.1e^{-0.014x}$, $R^2 = 0.991$

The titrated and predicted PFA concentrations in solution are shown in Figure 1.

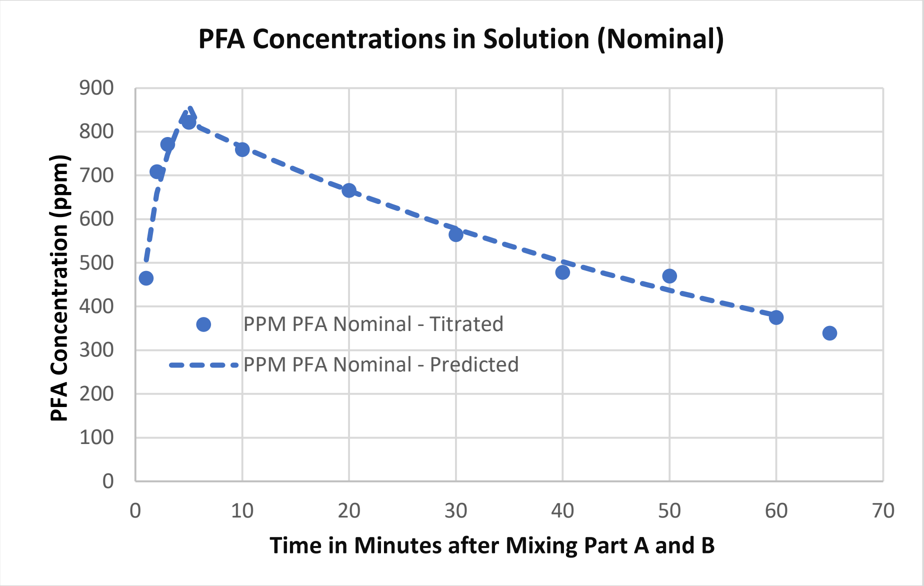

Figure 1. Titrated and Predicted PFA Nominal Concentrations

The PFA concentrations were averaged over 15-minute intervals to match the air sampling intervals from the available exposure study (MRID 51245501). These concentrations are as follows:

- For the time period 0 to 15 minutes, the PFA concentration is 746 ppm.
- For the time period 15 to 30 minutes, the PFA concentration is 639 ppm.
- For the time period 30 to 45 minutes, the PFA concentration is 518 ppm.
- For the time period 45 to 60 minutes, the PFA concentration is 420 ppm.

Inhalation MOE for Performic Acid (PFA)

The inhalation margin of exposure (MOE) for PFA was calculated using the PFA sample data and predicted PFA concentrations as outlined in Table 5. Although not stated in the study report, it is assumed that the cleaning was accomplished during the first two 15-minute air sampling periods; therefore, only the data for these sampling periods were used to calculate exposures. Given the sixty-minute use life of the product, it is also assumed that the product would be used to clean two rooms after which a fresh batch of product would be mixed. The overall average results for the first two 15-minute sampling intervals were averaged to obtain a 30-minute time-weighted average (TWA) for the time needed to clean one room. To calculate exposures during the cleaning of the first room using a freshly mixed batch, the exposure data were not adjusted for solution concentration. To calculate exposures for the second room that was cleaned, the exposure data were adjusted using the results of the titration testing to account for the decline in PFA solution concentrations. An 8-hour TWA was obtained by assuming that 15 rooms were cleaned per day (8 using a fresh batch) and assuming zero exposure for the remaining portion of the day to allow for non-cleaning activities such as set up time and travel between rooms. The MOE is 110 and not of concern because it is greater than the LOC of 100.

Table 5. Occupational Handler Performic Acid Inhalation MOE

Time (minutes)	PFA in Water (ppm)	PFA in Air (ppm)
0 to 15	746	0.083
15 to 30	639	0.037
0 to 30 Minute Average[A]		0.060
30 to 45	518	0.058[B]
45 to 60	420	0.024[C]
30 to 60 Minute Average[D]		0.041
8 Hour Time Weighted Average (TWA)[E]		0.048
MOE[F]		110

A. 0 – 30 min Average (ppm) = [0 -15 min PFA in Air (ppm) + 15 – 30 min PFA in Air (ppm)] / 2

B. 30 – 45 min PFA in Air (ppm) = 0 – 15 min PFA in Air (ppm) *[(30 – 45 min PFA in water (ppm) / 0 – 15 min PFA in water (ppm)]

C. 45 – 60 min PFA in Air (ppm) = 15 – 30 min PFA in Air (ppm) *[(45 – 60 min PFA in water (ppm) / 15 – 30 min PFA in water (ppm)]

D. 30 to 60 min Average (ppm) = [30 to 45 min PFA (ppm) + 45 to 60 min PFA (ppm)] / 2

E. 8-hour TWA (ppm) = [(0 to 30-min Average (ppm) * 30 min/room * 8 rooms /day) + (30 – 60 min Average (ppm)*30 min/room * 7 rooms/day)] / 480 min/day

F. MOE = 8 Hour HEC (5.4 ppm) / 8 Hour TWA (ppm)

Uncertainties in the Performic Acid MOE

There are several uncertainties related to the MOE calculated above for the exposures to PFA from the proposed use of glycerol formate. These uncertainties include the following:

- The MOE is based on a study where a mock cleaning of high-touch, non-floor surfaces in a bathroom was done to simulate exposures that would occur during the cleaning of a hospital room. The surface area cleaned (60 square feet) is probably larger than the non-floor surfaces that would be cleaned in a hospital room.
- The air sampling was conducted in the area near where the test subject was using the PFA solution rather than in the breathing zone of the test subject. This was done because air sampling in the breathing zone, where the sampling pump and sampling media are attached to the test subject, would have triggered the requirements of the Human Studies Rule. Depending upon the air currents present in the room in relation to the position of the test subject and air sampling device, the air concentrations could have been lower or higher in the breathing zone. It is for this reason that breathing zone air samples are preferred for assessing worker exposures while area samples are preferred for assessing exposure sources.
- The measured air concentrations were similar for both ventilation rates. This is unexpected as the ventilation rate is a driving variable for many exposure estimation models. There are two potential reasons for this:

1. The air concentrations were measured as area samples.
2. The air concentrations were measured under the supply diffuser. This diffuser operated as intended (i.e., the diffuser plates cause the air to be deflected sideways) when the system was operating at 6.8 ACH, which is likely the design airflow. When the airflow was reduced to 1.1 ACH, which is much less than the design airflow, the air was not deflected by the diffuser plates and was discharged downward through the center of the diffuser.

Some of the above uncertainties, such as the area cleaned, suggest that exposures could have been overestimated by the submitted study, while other uncertainties such as the ventilation rate suggest that the exposures could have been underestimated. It is not known if the uncertainties regarding the use of area samples suggest that exposures could have been overestimated or if they could have been underestimated.

Comparison to Hydrogen Peroxide Measurements

Although the use of hydrogen peroxide in the proposed PFA product is not considered a new use, air samples for hydrogen peroxide were collected during the PFA exposure study. The results of these samples, when compared to the NIOSH air sampling data that were included in the Peroxy Compounds DRA (US EPA, 2022), suggest that the PFA exposure study did not underestimate exposure. Using the hydrogen peroxide results with the same assumptions that were used for the PFA results (with the exception that adjustments for the titration data are not needed because hydrogen peroxide is more stable than PFA) to calculate exposure, the 8-hour TWA is 0.34 ppm as shown in Table 6.

Table 6. Hydrogen Peroxide Exposure Based on MRID 51245501

Chemical Assessed	0 to 15 Minute Overall Average^{A}	15 to 30 Minute Overall Average^{A}	30 Minute TWA^{B}	8-Hour TWA^{C}
Hydrogen Peroxide	0.43 ppm	0.28 ppm	0.36 ppm	0.34 ppm

A. Overall average for both ventilation rates.
B. 30-Minute TWA (ppm) = [0 to 15 Minute Overall Average (ppm) + 15 to 30 Minute Overage Average (ppm)] / 2
C. 8-hour TWA (ppm) = [30-minute TWA (ppm) * 30 minutes/room * 15 rooms /day] / 480 minutes/day

In the Peroxy Compounds Revised DRA (US EPA, 2022), the inhalation exposures for hydrogen peroxide were calculated using an average breathing zone concentration of 83 ppb that was from two Human Health Evaluation (HHEs) from NIOSH and adjusting the results for the application rate. If this method were used for the proposed PFA product, which has an application rate of 1.1% for hydrogen peroxide, the resulting 8-hour TWA would be 0.13 ppm as shown in Table 7. Since this 8-hour TWA is less than the 8-hour TWA of 0.34 ppm that was calculated based on the data from the PFA study, it can be concluded that the PFA study did not underestimate exposure as compared to the NIOSH data.

Table 7. Hydrogen Peroxide Exposure Based on the Peroxy Compounds Revised DRA

Scenario	Product	Application Rate (% H_2O_2)	8-Hour TWA (ppm)
Wipe Application Hospital	1677-237[A]	0.73	0.083[C]
	Glycerol formate[B]	1.1	0.13[D]

A. Product used in the NIOSH HHEs. Contains 27.5% H_2O_2 and 5.8% PAA and was diluted to 3 liquid ounces per gallon.
B. Glycerol formate product after mixing with Part B to yield a solution containing 1.1% H_2O_2.
C. Average breathing zone air concentration (83 ppb, n = 105) from NIOSH (2018) and NIOSH (2019).
D. NIOSH value adjusted for the application rate [*i.e.*, (1.1% H_2O_2 / 0.73% H_2O_2) x (0.083 ppm @ 0.73% H_2O_2)] = 0.13 ppm

3.11 Residential Exposure/Risk Characterization

There is the potential for residential bystander (i.e., patient) inhalation exposure to PFA during the application of the proposed product. The patient could be exposed to the product if it is used in occupied rooms. The 24-hour time weighted average (TWA) exposure for a patient occupying a room during and after treatment was calculated as shown in Table 8. The air concentration data for the first 60 minutes from Table 3 was used to represent the first hour of exposure and one half the LOD of 0.003 ppm was used to represent the remaining 23 hours of exposure. The air concentration data was not corrected using the titration data. Based on the 24-hour TWA of 0.003 ppm, the MOE is 600 which is not of concern because it is greater than the LOC of 100. For this assessment, the air concentrations are utilized, and no dose calculations are needed to account for population subgroups including children.

Table 8. Residential Bystander Patient Room Performic Acid Inhalation MOE

Time (minutes)	Exposure	PFA in Air (ppm)
0 to 15	Room Being Treated	0.083
15 to 30	Room Being Treated	0.037
30 to 45	Following Treatment	0.020
45 to 60	Following Treatment	0.004
60 Minute Average[A]		0.036
1380 Minute Average[B]		0.0015
24 Hour Time Weighted Average (TWA)[C]		0.003
MOE[D]		600

A. 60 min Average (ppm) = [0 -15 min PFA in Air (ppm) + 15 – 30 min PFA in Air (ppm) + 30 to 35 Minute PFA in Air (ppm) +(45-to-60-minute PFA in Air (ppm)] / 4
B. The PFA air concentration for the remainder of the day is assumed to be one half the LOD of 0.003 ppm
C. 24-hour TWA (ppm) = [(60-min Average (ppm) *60 Minutes) + (1380 minute Average (ppm) * 1380 minutes] / 1440 minutes
D. MOE = 24 Hour HEC (1.8 ppm) / 24 Hour TWA (ppm)

3.12 Human Health Incidents

Glycerol formate is a proposed new active ingredient; therefore, there are no human health incidents. However, since the primary exposures are to PFA, which can be produced by other currently registered chemicals (i.e., hydrogen peroxide and formic acid), the Incident Data System (IDS) was searched for incidents relevant to the proposed uses of glycerol formate. These incidents are for products where the active ingredient is formic acid or hydrogen peroxide, and it is not clear how the formic acid and hydrogen peroxide uses that resulted in the incidents reported relate to the proposed glycerol formate use patterns and resulting exposure.

Formic Acid
Based on a search of the IDS for formic acid from April 2019 to April 2024. No incidents were reported.

Hydrogen Peroxide
Based on a search of the IDS for all peroxy compounds from April 2019 to April 2024, there were 109 discrete reports. Of these, there were no reported deaths, and 10 incidents were reported as “major.” Major incidents included chemical burns and respiratory symptoms.

In addition to the incidents reported in the IDS, hospital employee complaints of respiratory irritation were investigated by the National Institute for Occupational Safety and Health (NIOSH) in two Health Hazard Evaluations (HHEs). Both these HHEs were conducted at hospitals where a peroxy product (OxyCide, EPA Reg. No. 1677-237), which contains 27.5% hydrogen peroxide and 5.8% peracetic acid, was diluted at the rate of 3 ounces per gallon and applied via wipes to clean and disinfect hard surfaces. In HHE 2015-0053-3259 (NIOSH, 2018), NIOSH found that increased exposure to hydrogen peroxide, peracetic acid and acetic acid vapors were associated with increases in acute work-related nasal and eye symptoms and increased shortness of breath on level ground reported by cleaning staff. In HHE 2017-0114-3357 (NIOSH, 2019), NIOSH reported similar findings regarding increased exposure to hydrogen peroxide, peracetic acid or acetic acid and symptoms reported by hospital staff. In both HHEs, NIOSH provided recommendations to reduce exposure. These recommendations included calibrating the product dispensers to properly dilute the product to maintain a pH of 2.7 to 4.0 and minimizing the use of the product in non-patient care areas.

4 ENVIRONMENTAL RISK ASSESSMENT

As stated previously, primary exposure is to PFA, formed by the reaction of glycerol formate and hydrogen peroxide. PFA degrades very rapidly to formate/formic acid and hydrogen peroxide (which in turn rapidly degrades into oxygen and water) and is expected to result in limited environmental exposure. Therefore, risks to nontarget terrestrial and aquatic organisms from PFA during antimicrobial uses of glycerol formate are expected to be negligible.

If any environmental exposure to glycerol formate occurs (e.g., in the PFA solution, which initially contains 1% glycerol formate, or in cases of spills), glycerol formate hydrolyzes rapidly to glycerol and formate/formic acid. Should any glycerol formate be released into the environment, glycerol formate and its degradates have low persistence and are expected to result in limited environmental exposure as well.

4.1 Environmental Fate

Performic Acid

Various environmental fate guideline studies have been submitted for glycerol formate, but none were submitted for PFA, the final active biocide form. However, public literature data are available for PFA. Given its vapor pressure (77.7 mmHg at 25°C, i.e., $\geq 10^{-6}$ mmHg criteria[3]) and Henry's Law Constant ($<10^{-5}$ atm-m^3/mol; i.e., $\geq 10^{-7}$ atm-m^3/mol criteria[4]) from Table 1, PFA is expected to volatilize readily from dry surfaces and aqueous solutions. Hydrolytic half-lives were estimated to be 4 minutes at pH 7 and 0.4 minutes at pH 8 (Mill *et al.*, 1987). Additionally, a white paper on PFA in oilfield uses indicates that PFA is highly reactive with metals and organic matter, and it is expected to degrade very rapidly to formate/formic acid and hydrogen peroxide in the environment; the observed half-lives of PFA were <6 minutes in produced water and ~60 minutes in distilled water (MRID 50705607; reviewed in US EPA, 2019b[5]). As a result, no significant exposure is expected for PFA in the environment, and no environmental fate data are therefore required.

EPA assembled a partial read-across for PFA, peracetic acid (PAA), and hydrogen peroxide and determined that PFA may generally be grouped with the peroxy compounds (Appendix C).

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[3] 10^{-6} mmHg is the general criteria for volatility from dry, non-adsorbing surfaces, based on OCSPP 835.6100, US EPA (2013), and US EPA (2021).

[4] 10^{-7} atm-m^3/mol is the general criteria for volatility from wet surfaces (*i.e.*, water and moist soil), based on OCSPP 835.6100, US EPA (2013), and US EPA (2021).

[5] US EPA (2019) erroneously reported the half-life to be 15-30 minutes in deionized water. The study (MRID 50705607) indicates the half-life to be ~60 minutes in distilled water (not deionized water).

More details on the fate profile for the peroxy compounds can be found in the Peroxy Compounds DRA (US EPA, 2022), which assesses risk of exposure from hydrogen peroxide, PAA, peroxyoctanoic acid, and sodium percarbonate. According to the Peroxy Compounds DRA (US EPA, 2022), peroxy compounds readily hydrolyze and rapidly break down to hydrogen peroxide (which in turn rapidly degrades into oxygen and water upon contact with organic matter) and respective associated acid/salt compounds. Furthermore, hydrogen peroxide had a half-life of 2 minutes in wastewater treatment plants (WWTPs) with an estimated removal of over 99% during wastewater treatment, biodegrading to water and oxygen prior to discharge from WWTPs (EU, 2003, as cited in US EPA, 2022).

Glycerol Formate

Although primary exposure is expected to be to PFA, this section also discusses the environmental fate data for the proposed new a.i., glycerol formate, as the registrant indicated that the final PFA solution will initially contain 1% glycerol formate (MRID 50864540).

Glycerol formate, in this risk assessment, is comprised of glycerol monoformate, diformate, and triformate, with the triformate species being the predominant species. Based on Table 1, glycerol formate has a high vapor pressure of 0.219 mmHg (i.e., $\geq 10^{-6}$ mmHg criteria[3]), indicating that it will volatilize from dry surfaces. Volatility from wet surfaces, on the other hand, may be limited due to low estimated Henry's law constant of $<10^{-8}$ atm·m^3/mol (i.e., $<10^{-7}$ atm-m^3/mol criteria[4]).

As indicated in Table 9 below, hydrolysis of glycerol formate at 25°C is rapid with a half-life of $\leq$1.88 days at pH 4, with more rapid degradation at higher pH values ($\geq$pH 7). The hydrolytic instability is consistent with the chemical structure containing hydrolyzable ester groups. Due to its structural similarity with glycerol triacetate (or more commonly called triacetin, CAS No. 102-76-1), which hydrolyzes in successive steps to diacetin, monoacetin, and finally acetate (or acetic acid) plus glycerol, glycerol formate (as the tri-ester form) is expected to hydrolyze similarly to diformate, monoformate, and finally formate/formic acid plus glycerol (MRID 51054201; OECD, 2002; Yamasaki, 1920). The major degradation products in the end are expected to be glycerol and formate/formic acid. With a log K_{ow} of -0.84, glycerol formate is not expected to significantly bioaccumulate in fish (Table 1).

Persistence in aquatic and soil environments is low for glycerol formate and its degradates. Glycerol formate is mobile to highly mobile in soil, based on a log K_{oc} value of <1.25 in a non-guideline high-performance liquid chromatography (HPLC) study. However, contact with soil is expected to be insignificant, as the proposed use pattern of glycerol formate does not result in direct or indirect (through residues in land-applied sludge) applications to soil, and glycerol formate rapidly hydrolyzes to glycerol and formic acid/formate. Glycerol formate is also readily biodegradable, and open literature (as cited in MRIDs 50864534, 50864535, and 50864540) indicates the degradates are also biodegradable under aerobic and anaerobic conditions and are metabolized by microorganisms in many important biochemical processes.

Activated sludge respiration inhibition (ASRI) data were submitted for glycerol formate and demonstrate a high half-maximal inhibitory concentration (IC_{50} = 198.8-514.6 mg/L; i.e., >20 mg/L), indicating the unlikelihood of any adverse effects of glycerol formate on activated sludge microorganisms.

Table 9 below summarizes the environmental fate guideline studies submitted for glycerol formate and PFA.

Table 9. Environmental Fate Data for Glycerol Formate and Performic Acid (PFA)

Guideline No. (GLN)	Guideline Study Name	Glycerol Formate	Performic Acid (PFA)
835.2120	Hydrolysis	At 25°C: pH 4: $DT_{50} \leq$ 1.88 days pH 7: $DT_{50} \leq$ 0.0477 days (69 min) pH 9: DT_{50} < 0.0104 day (15 min) (MRID 50864530; acceptable; US EPA, 2020)	pH 7: 4 minutes pH 8: 0.4 minutes (Mill *et al.*, 1987) Produced water: <6 minutes Distilled water: ~60 minutes (MRID 50705607; US EPA, 2019b)
835.3110	Ready Biodegradability	Readily biodegradable (80% CO_2 evolution after 10 days; 83-86% CO_2 evolution on days 14-28); non-toxic to WWTP microorganisms (MRID 50864533; acceptable; US EPA, 2020)	Sludge biodegradation: No data, but DT_{50} is expected to be faster than for PAA) (PAA DT_{50} = 3 minutes; EU, 2015, as cited in US EPA, 2022)
850.3300	Activated Sludge Respiration Inhibition (ASRI)	IC_{50} = 198.8-514.6 mg/L (*i.e.*, >20 mg/L); not expected to be toxic to WWTP microorganisms (MRID 50864532; supplemental; US EPA, 2020)	No data
Non-guideline	Estimation of Adsorption Coefficient (K_{oc}) by HPLC	Log K_{oc} <1.25; mobile to highly mobile in soil (MRID 50864536; satisfies OCSPP 835.1230 Adsorption/Desorption guideline requirement)	No data

DT_{50} = half-life; WWTP = wastewater treatment plant; IC_{50} = half-maximal inhibitory concentration.

Although PFA may be released into the waste stream after use in sinks or near drains, it will rapidly hydrolyze to formic acid/formate and hydrogen peroxide (which in turn rapidly degrades into oxygen and water upon contact with organic matter), minimizing any exposure to wastewater.

4.2 Environmental Fate Uncertainties

The information provided by the submitted studies and the open literature were determined to be sufficient for this risk assessment on glycerol formate and PFA.

4.3 Degradates of Potential Concern

PFA is extremely reactive with metals and organic matter and must be generated on-site by mixing glycerol formate and hydrogen peroxide. As a highly unstable strong oxidant, PFA degrades rapidly in the environment to formate/formic acid and hydrogen peroxide (which in turn rapidly degrades into oxygen and water upon contact with organic matter) and presents minimal concerns to the environment.

If any environmental exposure (terrestrial or aquatic) to glycerol formate occurs (e.g., in the PFA solution, which initially contains 1% glycerol formate, or in cases of spills), glycerol formate degrades rapidly to glycerol and formate/formic acid. These degradation products present minimal concerns to the environment. Glycerol is found in triglycerides, which are main constituents of vegetable and animal fats and oils and is part of many important biochemical processes. Formic acid is a simple carboxylic acid that is ubiquitous in the environment and is found in plants, animals, soil, surface water, and the atmosphere. It is also an important metabolic intermediate. Formic acid/formate is a common product of bacterial fermentation and does not accumulate under oxic conditions (Lim *et al.*, 2014), suggesting it transforms readily. Oxidation of formic acid/formate under aerobic conditions yields carbon dioxide and water. In addition, both glycerol and formic acid have been classified by the US FDA as generally recognized as safe (GRAS).

4.4 Ecological Effects

The registrant has submitted five guideline studies as well as waiver requests for the additional ecotoxicity data for glycerol formate, as outlined in the 40 CFR §158W. All studies have been reviewed, and all waiver requests were granted. The selected ecotoxicity endpoints are outlined in the table below and indicate glycerol formate is practically non-toxic to birds and freshwater invertebrates, and slightly toxic to freshwater fish.

Table 10. Ecological Effects Endpoints Selected for Glycerol Formate

Guideline (OCSPP Guideline No.)	Surrogate Species	Study Results	Acute Toxicity Category	MRID/ Study Classification
Acute avian oral (850.2100)	Northern Bobwhite (*Colinus virginianus*)	14-day LD_{50} = >2,000 mg a.i./kg bw (nominal)	Practically Non-toxic	50864522 Acceptable
Acute freshwater invertebrates (850.1010)	Water Flea (*Daphnia magna*)	48-hr EC_{50} >100 mg/L (nominal)	Practically Non-Toxic	50864523 Supplemental (Qualitative)
Acute freshwater fish (850.1075)	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50} >72 mg a.i./L (mean measured)	Slightly Toxic	50864524 Acceptable
Aquatic nonvascular plant (850.4500)	freshwater green alga, *Raphidocelis subcapitata*	IC_{50} = 62 mg/L (nominal) Yield and Area Under the Growth Curve	--	50864526 Supplemental (Qualitative)
Aquatic nonvascular plant-cyanobacteria (850.4550)	Cyanobacteria, *Anabaena flos*-aquae	IC_{50} = 49 mg/L (nominal) Area under the growth curve	--	50864527 Supplemental (Qualitative)

Due to the instability of PFA and the proposed indoor use of the glycerol formate end-use product, the potential for environmental exposure to PFA is expected to be limited. Therefore, no ecotoxicity data on PFA has been submitted to the Agency. That said, similar to human health toxicity, PFA may generally be grouped with the peroxy compounds. The toxicity profile of peroxy compounds indicates that they are generally more toxic to aquatic organisms than glycerol formate. Depending on the compound tested and whether it was tested in the form of a product or technical grade active ingredient (TGAI), peroxy compounds are slightly toxic to birds, practically non-toxic to bees, practically non-toxic to highly toxic to freshwater fish, moderately toxic to estuarine/marine fish, slightly to highly toxic to freshwater invertebrates, moderately to highly toxic to estuarine/marine invertebrates, and moderately to highly toxic to bivalves. In addition, decreases in cell density in vascular plants and non-vascular aquatic plants above 1 mg/L were observed. For more details on the ecotoxicity of peroxy compounds, see the registration review documents within docket EPA-HQ-OPP-2009-0546 at www.regulations.gov.

4.5 Ecotoxicity Uncertainties

The registrant has satisfied all ecotoxicity data required within the 40 CFR §158W for the indoor use pattern of the proposed new a.i. with either an acceptable waiver request or guideline study. No additional data are needed at this time to support the risk assessment.

4.6 Aquatic Exposure

No aquatic exposure modeling was performed in this assessment because all registered antimicrobial uses of glycerol formate are considered indoor with a low likelihood of environmental exposure. In the event that PFA is released into a waste stream, the Agency believes that it will volatilize and degrade rapidly and, therefore, pose limited exposure potential within wastewater treatment plants, surface water, and groundwater. Thus, no adverse impacts to nontarget plants and wildlife are expected, and only a qualitative ecological risk assessment was conducted for the antimicrobial uses of glycerol formate.

4.7 Ecological Risk Characterization

Based on the proposed indoor use pattern, high volatility, and rapid degradation in the environment, terrestrial and aquatic exposures to PFA are not expected when the product is used according to label specification. As a result, the Agency does not anticipate any adverse effects to nontarget organisms from the indoor uses of the proposed new a.i., glycerol formate.

4.8 Ecological Incident Data

Glycerol formate is the proposed new active ingredient; therefore, there are no ecological incidents. However, since the primary exposures are to PFA, which can be produced by other currently registered chemicals (i.e., hydrogen peroxide and formic acid), the Incident Data System (IDS) was searched for incidents relevant to the proposed uses of glycerol formate.

Hydrogen peroxide
The IDS was searched on April 26, 2024, using the search terms “hydrogen peroxide” and PC code “000595”. The incidents noted did not involve registered antimicrobial products.

Formic acid
The IDS was searched on April 26, 2024, using the search term “formic acid”. The incidents noted did not involve registered antimicrobial products.

5 LISTED SPECIES OF CONCERN

The uses for glycerol formate, based on the proposed label (DuoGuard RTU, Reg. No. 1677-EAA), include cleaning and disinfecting hard, non-porous surfaces (e.g., counters and tables, toilets, showers, etc.) and for sanitizing soft surfaces (e.g., bedding, blankets, curtains, towels, etc.) at healthcare facilities (e.g., hospitals, medical premises, nursing homes, etc.) using wipes and cloths, and are confined to indoor environments where releases could occur via down-the-drain to wastewater treatment plants and subsequently released to surface waterbodies. Based on the environmental fate characteristics, however, the potential for exposure to aquatic organisms is expected to be negligible. The potential for direct exposure to terrestrial vertebrates and invertebrates from the indoor use of glycerol formate is expected to be negligible as well. No reasonable expectation of discernible direct or indirect effects to threatened or endangered species or the designated critical habitat for such species is expected from the indoor antimicrobial use of glycerol formate. Therefore, the Agency is making a "no effect" determination under the Endangered Species Act (ESA) for all listed species and designated critical habitats for such species based on the expected negligible exposure from the antimicrobial use of glycerol formate.

Based on the label, two parts (Part A, 99.7% glycerol formate and Part B, 1.1% hydrogen peroxide) are mixed together to form PFA, which is designed to be efficacious for 60 minutes after mixing. According to the registrant, this final solution will still contain 1% glycerol formate (MRID 50864540). Glycerol formate is a blend of three ester forms: glycerol monoformate, glycerol diformate, and glycerol triformate. In an aqueous environment, glycerol formate will rapidly degrade (half-lives of <0.05 day in neutral and alkaline waters and 1.9 days in acidic waters) to glycerol and formate/formic acid (MRID 50864530). Open literature (as cited in MRID 50864540) indicates that these degradates are biodegradable under aerobic and anaerobic conditions. Glycerol is found in triglycerides, which are the main constituents of vegetable and animal fats and oils and is part of many important biochemical processes (e.g., glycerol is an intermediate in carbohydrate and lipid metabolism). Formic acid is a simple carboxylic acid that is ubiquitous in the environment and is found in plants, animals, soil, surface water, and the atmosphere; it is also an important metabolic intermediate. Formic acid/formate is a common product of bacterial fermentation and does not accumulate under oxic conditions (Lim *et al*., 2014), suggesting it transforms readily. Oxidation of formic acid or formate under aerobic conditions yields CO_2 and water. In addition, both glycerol and formic acid have been classified by the US FDA as generally recognized as safe (GRAS). With a log K_{ow} of -0.84, any glycerol formate present in a waterbody is not expected to significantly bioaccumulate in fish (MRID 50864502).

In aquatic environments, glycerol formate is considered practically nontoxic to aquatic invertebrates (EC_{50} > 100 mg a.i./L), slightly toxic to freshwater fish (LC_{50} > 72 mg a.i./L) and has an IC_{50} of 49 mg a.i./L for aquatic nonvascular plants based on area under the growth curve (MRIDs 50864523, 50864524, and 50864527, respectively). While guideline toxicity studies are not available for PFA, there is limited information available in the open literature for similar chemistries that can be used to inform the toxicity of PFA. A 24-hour median tolerance limit (TLm; the concentration lethal to 50% of the fish) of 175 mg/L formic acid was derived for bluegill sunfish, *Lepomis macrochirus* (Dowden and Bennett, 1965). Metoc (2003) reported results of acute toxicity tests of various formate salts to juveniles and larvae of the marine fish, turbot (*Scothphthalmus maximus*). The LC_{50} for juvenile turbot was 260 mg/L (96-hour) cesium formate, and for larvae was 1,400 mg/L (48-hour) cesium formate. For the potassium and sodium salts of formate, 96-hour LC_{50} values for juvenile turbot were greater than 1,000 mg/L.

PFA, the active biocide, is extremely reactive with metals and organic matter. Given its vapor pressure (77.7 mmHg at 25°C) and Henry's Law Constant ($<10^{-5}$ atm-m^3/mol), PFA is expected to volatilize readily from dry surfaces and aqueous solutions. Hydrolysis half-lives at pH 7 and 8 indicate were estimated at 4 and 0.4 minutes, respectively (Mill *et al.*, 1987). As a highly unstable strong oxidant, PFA degrades rapidly in the environment to formate/formic acid and water and presents minimal concerns to the environment as well. The Agency assembled a partial read-across for PFA, peracetic acid, and hydrogen peroxide and determined that PFA may generally be grouped with the peroxy compounds. The toxicity profile of peroxy compounds indicates that they are generally more toxic to aquatic organisms than glycerol formate. Depending on the compound tested and whether it was tested in the form of a product or technical grade active ingredient (TGAI), peroxy compounds are practically non-toxic to highly toxic to freshwater fish, moderately toxic to estuarine/marine fish, slightly to highly toxic to freshwater invertebrates, moderately to highly toxic to estuarine/marine invertebrates, moderately to highly toxic to bivalves, and toxic to vascular and non-vascular aquatic plants at levels above 1 mg/L.

As discussed above, based on the proposed use patterns, which are all indoor uses, exposure to nontarget organisms is expected to be negligible. Additionally, based on (1) the rapid transformation of glycerol formate and hydrogen peroxide to PFA, (2) the rapid degradation of PFA to formate/formic acid and water, (3) the rapid degradation of glycerol formate to glycerol and formic acid (in the event that any glycerol formate is washed down the drain), (4) the relatively low toxicity profile of glycerol formate, PFA, and its degradates, and (5) the presence of glycerol and formic acid in natural systems in the environment, any potential exposure concentrations of glycerol formate and its degradates would be expected to be well below the effects levels. As a result, the Agency is making a "no effect" determination for glycerol formate under the ESA for all listed aquatic species and designated critical habitats for such species. Additionally, as the potential for exposure to terrestrial vertebrates, invertebrates, and plants from the indoor use of glycerol formate is expected to be negligible, the Agency believes there will not be any discernible direct or indirect effects to prey items or habitat for terrestrial vertebrates, invertebrates, and plants. As a result, the Agency is making a "no effect" determination for glycerol formate under the ESA for all listed terrestrial species and designated critical habitats for such species. The Agency has therefore concluded that consultation with the US Fish and Wildlife Service and the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service under ESA section 7(a)(2) is not required.

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7 APPENDIX A. Acute Toxicology Profile

The acute toxicity studies for hydrogen peroxide and peracetic acid, are summarized in Tables A1 and A2. The technical forms of the peroxy compounds are corrosive to the skin at high concentrations and irritating to the skin (Toxicity Category I). All the peroxy compounds are strong eye irritants (Toxicity Category I). Acute oral toxicity is Toxicity Category III and appears to be less of a concern for peroxy compounds than other exposure routes for pesticidal use. Exposure via the inhalation route is moderately irritating to the lungs for hydrogen peroxide (Toxicity Category II). Dermal sensitization studies for technical products were waived for hydrogen peroxide and peracetic acid due to the strong irritant properties at high concentrations (US EPA, 1993). Given the greater reactivity and acidity of PFA compared to H_2O_2 and PAA, respectively, PFA has greater toxicity potential compared to the other peroxy compounds. In this specific case, performic acid is more likely to be similar to peracetic acid.

Table A1. Acute Toxicity Studies for Hydrogen Peroxide[1]

Guideline No./Study Type	Results	Toxicity Category
870.1100 Acute oral toxicity (mouse)	LD_{50} = 2000 mg/kg	III
870.1200 Acute dermal toxicity (rat)	LD_{50} = 4060 mg/kg	III
870.1300 Acute inhalation toxicity (mouse)	LC_{50} = 227 µL/L (ppm) 0.32 mg/L	II
870.2400 Acute eye irritation (rabbit)	Severe irritation	I
870.2500 Acute dermal irritation (rabbit)	Corrosive	I
870.2600 Skin sensitization	Waived	

[1] Sax *et al.* (1989), as cited in US EPA (1993)

Table A2. Acute Toxicity Studies for Peracetic Acid[1]

Guideline No./Study Type	Results	Toxicity Category
870.1100 Acute oral toxicity (rat)	LD_{50} = 1540 mg/kg	III
870.1200 Acute dermal toxicity (rabbit)	LD_{50} = 1410 mg/kg	II
870.1300 Acute inhalation toxicity (rat)	LC_{50} = 0.450 mg/L	II
870.2400 Acute eye irritation (rabbit)	Severe irritation	I
870.2500 Acute dermal irritation (rabbit)	Corrosive	I
870.2600 Skin sensitization	Waived	

[1] Sax *et al.* (1989), as cited in US EPA (1993)

8 APPENDIX B. Ecotoxicity Profile

Table B1. Ecological Effects Endpoints for Studies Performed on Products with Peracetic Acid and Hydrogen Peroxide in Equilibrium. Endpoints Determined on the Whole Product (PC Codes 063201 and 000595) (Summarized)

Receptor Group	Surrogate Species	Toxicity Endpoint (Product)	Percent a.i. in Product	Toxicity Category of the Product	Reference
Avian Acute Oral (850.2100)	Mallard Duck (*Anas platyrhynchos*)	LD_{50} = 619 mg Product/kg-bw	% a.i. not given.	Slightly Toxic	MRID 47477201 (Acceptable) Gavage
Freshwater Fish Acute (850.1075)	Rainbow trout *(Salmo gairdneri)*	96-Hr LC_{50} = 13.4 mg/L Product	15% peracetic acid 14% H_2O_2 28% acetic acid	Slightly Toxic	MRID 46833606 (Acceptable) Static Renewal
Estuarine/Marine Fish Chronic (850.1400)	Inland Silverside *(Menidia beryllina)*	7-day LC_{50} = 35.6 mg/L Product	15% peracetic acid, 14.3% H_2O_2	N/A	MRID 46833604 (Acceptable) Static Renewal
Freshwater Invertebrates Acute (850.1010)	Daphnid (*Daphnia magna*)	48-Hr LC_{50} = 3.3 mg/L Product	15% peracetic acid, 14% H_2O_2 28% acetic acid	Moderately Toxic	MRID 46833603 (Acceptable) Static
Estuarine/Marine Invertebrates Acute (850.1035)	Mysid (*Americamysis bahia)*	96-Hr LC_{50} > 3.0 mg/L Product	% a.i. not given.	Moderately Toxic	MRID 47477203 (Acceptable) Static
Bivalve acute toxicity test (embryo larval) (850.1055)	Mediterranean Blue mussel *(Mytilus galloprovincialis)*	48-Hr EC_{50} = 3.7 mg/L Product	15% peracetic acid 24% H_2O_2	Moderately Toxic	MRID 46833604 (Acceptable) Static
	Blue mussel *(Mytilus galloprovincialis)*	48-Hr EC_{50} = 3.68 mg/L product	15% peracetic acid 14% H_2O_2 26.5% acetic acid	Moderately Toxic	MRID 46833605 (Acceptable) Static
Aquatic plant toxicity test using Lemna spp.	Duckweed (*Lemna gibba*)	7-day EC_{50} = 230 mg/L Product (nominal) (biomass)	12.08% peracetic acid 19.44% H_2O_2	N/A	MRID 46966604 (Core) Static

Receptor Group	**Surrogate Species**	**Toxicity Endpoint (Product)**	**Percent a.i. in Product**	**Toxicity Category of the Product**	**Reference**
(850.4400)	Duckweed (*Lemna gibba*)	7-day EC_{50} = 81 mg/L Product (nominal) (frond density) 7-day EC_{50} = >200 mg/L Product (nominal) (growth rate) 7-day EC_{50} = 67 mg/L Product (nominal) (biomass)	% a.i. not given.	N/A	MRID 47477202 (Acceptable) Static Renewal
Alga toxicity (850.4500)	Marine Diatom (*Skeletonema costatum*, strain CCMP 1332)	Cell Density 96-Hr EC_{50} = 27 mg/L Product	12.12% peracetic acid 18.95% H_2O_2	N/A	MRID 46966607 (Core) Static
	Marine Diatom (*Skeletonema costatum*)	Cell Density 96-Hr EC_{50} = 19 mg/L Product	% a.i. not given.	N/A	MRID 47477204 (Supplemental) Static
	Freshwater Diatom (*Navicula pelliculosa,* strain 664)	Cell Density 96-Hr EC_{50} = 0.56 mg/L Product	12.12% peracetic acid 18.95% H_2O_2	N/A	MRID 46966605 (Core) Static
	Freshwater Diatom (*Navicula pelliculosa*)	Cell Density 96-Hr EC_{50} = 2.3 mg/L Product	% a.i. not given.	N/A	MRID 47477205 (Supplemental) Static
Cyanobacteria Toxicity (850.4550)	Cyanobacteria (*Anabaena flos-aquae*, strain 1444)	Cell Density 96-Hr EC_{50} = 1.5 mg/L Product	12.12% peracetic acid 18.95% H_2O_2	N/A	MRID 46966606 (Core) Static
	Cyanobacteria (*Anabaena flos-aquae*)	Cell Density 96-Hr EC_{50} = 0.21 mg/L Product	% a.i. not given.	N/A	MRID 47477206 (Supplemental) Static

Receptor Group	Surrogate Species	Toxicity Endpoint (Product)	Percent a.i. in Product	Toxicity Category of the Product	Reference
Terrestrial Seedling Emergence 850.4100 and 850.4225	Rice (*Oryza sativa* of the Japonica variety)	Percent emergence: EC_{50}= 4700 mg/kg Product Shoot length: EC_{50}> 6000 mg/kg Product Shoot dry weight (mg) EC_{50}= 6200 mg/kg Product	12.08% peracetic acid 19.44% H_2O_2	N/A	MRID 46966608 (Core) Static
	Red Rice (*Oryza sativa*)	NOEC = 60 mg/L Product (% emergence, shoot length, and shoot dry weight (mg))	15% peracetic acid 22% H_2O_2	N/A	46696003 (Acceptable) Static
Terrestrial Invertebrate-Honeybee Toxicity (850.3020)	Honey Bee (*Apis mellifera*)	96-hr LD_{50} > 25 µg product/bee or	Product was 2.05% peracetic acid 26.93% H_2O_2	Practically Non-Toxic	MRID 49801001 (Acceptable)

NA: Not applicable. Aquatic plants and chronic studies do not have a toxicity category.

9 APPENDIX C. Rationale for Grouping performic acid with the peroxy compounds

Glycerol formate is proposed for registration as an end-use product (DuoGuard RTU, EPA Reg. No. 1677-EAA) that is part of a two-part system with hydrogen peroxide (H_2O_2) to generate peroxyformic acid, or performic acid, (PFA) *in situ.* The resulting use solution contains 1% glycerol formate, 1.1% H_2O_2 and 800 ppm PFA, with a target PFA concentration of at least 300 ppm throughout the use life (up to 60 minutes) (MRIDs 50864504 and 52062301). There are presently no concerns for exposures to glycerol formate as it is contained in a closed system; therefore, this document will focus on PFA. The use solution containing PFA is applied for the purpose of disinfecting hard, non-porous surfaces and sanitizing soft surfaces using a cloth wipe for the purpose of disinfecting or sanitizing those surfaces. The product is proposed for use in hospital settings (e.g., hospitals, medical premises/equipment, and nursing homes/assisted living facilities). Based on the use pattern, the occupational handler exposures to healthcare workers are of greatest concern (i.e., highest amount handled, daily exposure, long-term duration). Exposure will be to PFA, which, according to the labels, remains efficacious for up to approximately 1 hour from the time the ingredients are combined. It is anticipated that workers will mix up and use several batches per day.

As mentioned previously, human health toxicity data are not available for PFA. In lieu of providing the toxicity data required for the proposed registration of a new active ingredient as outlined in 40 CFR §158.2230, the registrant provided a rationale for bridging PFA to the peroxy compounds for the purposes of conducting the risk assessment (MRID 51476901), but it was of limited utility. While the registrant provided a side-by-side comparison of the physical/chemical properties for PFA and PAA, the significance of these properties in driving toxicity was not sufficiently discussed in detail. Based on a partial read-across table (Table C1), the Agency determined that PFA is structurally similar to PAA, and to a lesser extent, H_2O_2, on the basis that all are oxidants with a highly reactive peroxy functional group. However, PFA is more reactive (i.e., less stable) than PAA, which in turn is more reactive than H_2O_2. The greater reactivity of PFA, and thus the greater potential for toxicity, compared to PAA and H_2O_2 adds more uncertainty to the toxicological endpoints used for PFA, which are already based on the limited toxicological data for H_2O_2.

In evaluating whether it is appropriate to bridge PFA to the other peroxy compounds, EPA relied on the OECD Guidance on Grouping of Chemicals (OECD, 2014). According to the guidance, “A chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity. The similarities may be based on the following:

- a common functional grouping
- common constituents or chemical classes
- an incremental and constant changes across the category
- the likelihood of common precursors and/or breakdown products, via physical or biological processes, which result in structurally similar chemicals."

The following sections present this analysis.

C.1 Ingredient Profile and Chemical Identity

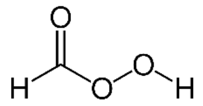
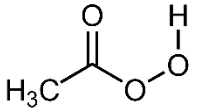
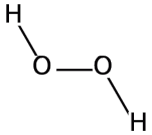
Glycerol formate is formed by the esterification reaction of glycerol with formic acid and is a blend of three ester forms: glycerol monoformate, glycerol diformate, and glycerol triformate. The triester form (glycerol triformate) is the predominant form in glycerol formate and drives the rapid production of performic acid. Glycerol formate reacts with hydrogen peroxide to form performic acid (PFA), the active biocide, as illustrated in the reactions below:

Glycerol triformate + H_2O_2 → performic acid + glycerol diformate
Glycerol diformate + H_2O_2 → performic acid + glycerol monoformate
Glycerol monoformate + H_2O_2 → performic acid + glycerol

In an aqueous environment, pure glycerol formate will rapidly degrade to glycerol and formate/formic acid. PFA, a highly unstable strong oxidant, will degrade rapidly in an aqueous environment to form formate/formic acid and hydrogen peroxide (H_2O_2); these degradates will subsequently degrade to carbon dioxide, water, and oxygen when stabilizing compounds are not present. It should be noted, however, that PFA is never in its pure state without stabilizers present. Peracetic acid (PAA), a structurally similar compound to PFA, will also rapidly degrade to form acetic acid and hydrogen peroxide and eventually to carbon dioxide, water, and oxygen. Table C1 below lists the chemical identities and physical properties of PFA (the active biocide), peracetic acid (PAA), and hydrogen peroxide.

Table C1. Chemical Identities and Physical Properties of PFA, PAA, and H_2O_2

Parameter	Performic Acid (PFA)	Peracetic Acid (PAA)	Hydrogen Peroxide (H_2O_2)
Molecular Weight (g/mol)	62.025	76.05	34.01
CAS Number	107-32-4	7921-0	7722-84-1
Molecular Formula	CH_2O_3	$C_2H_4O_3$	H_2O_2

Parameter	Performic Acid (PFA)	Peracetic Acid (PAA)	Hydrogen Peroxide (H_2O_2)
Molecular Structure			
SMILES Code	C(=O)OO	CC(=O)OO	OO
Density (g/cm^3)	1.34 (ChemSrc, 2021)	1.23 (NCBI, 2021b)	1.44 (NCBI, 2021a)
Melting Point (°C)	-46.2 (EPI-Suite, v4.11, est)	-0.2 (EPI-Suite, v4.11, exp)	-0.43 (EPI-Suite, v4.11, exp)
Boiling Point (°C)	127.5 (EPI-Suite, v4.11, exp)	110 (EPI-Suite, v4.11, exp)	152 (EPI-Suite, v4.11, exp)
Water Solubility @ 25°C (mg/L)	1×10^6 (EPI-Suite, v4.11, exp)	1×10^6 (EPI-Suite, v4.11, exp)	1×10^6 (EPI-Suite, v4.11, exp)
Dissociation Constant (pK_a)	7.1 (Rappoport, 2006, p. 698) 7.77 (NCBI, 2021c)	8.20 (NCBI, 2021b)	11.62 (NCBI, 2021a)
Vapor Pressure @ 25°C (mmHg)	77.7 (EPI-Suite, v4.11, est)	14.5 (EPI-Suite, v4.11, exp)	1.97 (EPI-Suite, v4.11, exp)
Henry's Law Constant ($atm \cdot m^3/mol$)	1.9×10^{-6} (EPI-Suite, v4.11, estimated by Bond SAR method)	2.14×10^{-6} (EPI-Suite, v4.11, exp)	7.04×10^{-9} (EPI-Suite, v4.11, exp)
Octanol-Water Partition Coefficient (log K_{ow})	-1.62 (EPI-Suite, v4.11, est)	-1.07 (EPI-Suite, v4.11, est)	-1.57 (EPI-Suite, v4.11, est)
O-O bond dissociation energy (kcal/mol)	48 (Bach *et al.*, 1996)	48 (Bach *et al.*, 1996) 38 (Kim and Huang, 2021)	50 (Bach *et al.*, 1996) 51 (Kim and Huang, 2021)
Lowest Unoccupied Molecular Orbital (LUMO) energy (eV)	No data Calculated eLUMO = 0.653 (QSAR Toolbox, v4.4.1)	0.25 (Kim and Huang, 2021) Calculated eLUMO = 0.675 (QSAR Toolbox, v4.4.1)	0.57 (Kim and Huang, 2021)
CompTox Dashboard similarity	No data	No stated similarity with PFA or H_2O_2	No stated similarity with PFA or PAA

All EPI-Suite values are from version 4.11, which includes the 2017 update files (US EPA, 2017a).
Exp = experimental data; est = estimated.

C.2 Environmental Fate Data for the Peroxy Compounds and PFA

No environmental fate guideline studies have been submitted for PFA. However, a white paper submitted by Nalco Champion (MRID 50705607) indicates that PFA is highly reactive with metals and organic matter, and it is expected to degrade very rapidly to formate/formic acid in the environment, with observed half-lives of <6 minutes in produced water and ~60 minutes in distilled water. Formic acid is a naturally-occurring component in many foods, a common intermediate in normal metabolism, and classified as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA). Formic acid/formate easily further degrades to carbon dioxide and water in the environment. More information about formic acid can be found in the Agency's public docket, EPA-HQ-OPP-2014-0105, available at www.regulations.gov. The available environmental fate data for PFA, PAA, H_2O_2, and the general peroxy acid (peracid) family are provided in Table C2.

Table C2. Environmental Fate Data for PFA and the Peroxy Compounds (PAA, H_2O_2, and Peroxy Acid Family)

Parameter	Performic Acid (PFA)	Peracetic Acid (PAA)	Hydrogen Peroxide (H_2O_2)	Peroxy Acid (Peracid) Family
Hydrolysis	DT_{50} observed to be <6 min in produced water and ~60 min in distilled water CAKE calculations: DT_{50} = 3.5 min in produced water and 82 min in distilled water (Above data based on MRID 50705607 for another PFA product (EPA Reg. No. 1677-269) that is generated onsite from formic acid and H_2O_2 (US EPA, 2019b) DT_{50} = 4 min at pH 7 and 0.4 min at pH 8 (Mill *et al.*, 1987) Expected degradates: formic acid and H_2O_2	DT_{50} = 46.7 hours at pH 4, 31.7 hours at pH 7, and 3.6 hours at pH 9 (EU, 2015, as cited in US EPA, 2022) DT_{50} = 8.3 hours (EPI-Suite, v4.11, est) Expected degradates: acetic acid and H_2O_2	Rapidly degrades to oxygen and water upon contact with organic matter (US EPA, 2022)	Expected to have same half-life as PAA and break down into H_2O_2 and organic component (R acid/salt form) (US EPA, 2022)
Sludge Biodegradation	No data (but DT_{50} expected to be faster than for PAA)	DT_{50} = 3 min (EU, 2015, as cited in US EPA, 2022)	DT_{50} = few seconds in sludge; few minutes to hours in municipal wastewater (EU, 2003, as cited in US EPA, 2022)	DT_{50} = <20 min (US EPA, 2022)
Air Dissipation	No data (but DT_{50} expected to be faster than for PAA)	DT_{50} = 20 min (US EPA, 2022)	DT_{50} = ~1 day (US EPA, 2022)	Varies but includes PAA range
Metabolites	Formic acid and H_2O_2 à finally to CO_2, water, and oxygen	Acetic acid and H_2O_2 à finally to CO_2, water, and oxygen	Water and oxygen	R acid form, water, and oxygen

As seen in Table C1, PFA has a shorter carbon chain length than PAA and thus is expected to be less stable (more reactive) than PAA. However, according to the registrant in the bridging rationale (MRID 51476901), reactivity is not directly correlated to oxidation potential, which is the property associated with cytotoxicity and irritation. Oxidation potential is correlated to bond dissociation energy instead. The registrant argues that PFA and PAA have similar oxidation potentials, due to identical O-O bond dissociation energies (48 kcal/mol; Bach *et al*., 1996; Table C2). Thus, the strength of reactions for both chemicals would be similar, and therefore, the toxicity data for PAA can be used for PFA.

The mechanism for oxidation of organic compounds by PAA and H_2O_2 (and similarly expected for PFA) is the transfer of an electrophilic oxygen atom from the peroxy compound to an electron-rich site on the organic compound. According to Kim and Huang (2021), PAA has a higher reactivity than H_2O_2, due to a weaker O-OH bond energy (38 kcal/mol, which the Agency notes is lower than the 48 kcal/mol calculated in Bach *et al*., 1996) and a lower Lowest Unoccupied Molecular Orbital (LUMO) energy (0.25 eV), compared to H_2O_2 (bond energy of 51 kcal/mol and LUMO energy of 0.57 eV) (Table C2). For instance, H_2O_2 readily reacts with thiol groups but at rates slower than those for PAA. The apparent second-order rate constant (k_{app}) value for L-cysteine at pH 7, 10 mM phosphate was 0.57 $M^{-1}\cdot s^{-1}$ when reacted with H_2O_2 and >580 $M^{-1}\cdot s^{-1}$ with PAA. The k_{app} values with PAA are at least 2 orders of magnitude higher than with H_2O_2. Using the OECD QSAR Toolbox, v4.4.1 (OECD, 2020), both PFA and PAA have comparable calculated eLUMO energies (0.653 eV and 0.675 eV, respectively). The lower the LUMO energy is, the more easily a chemical compound will accept electrons (i.e., become a stronger oxidizer). Therefore, based on the calculated eLUMO energies, PFA is expected to have comparable reactivity with PAA. However, both PFA and PAA may be two orders of magnitude more reactive than H_2O_2; this adds more uncertainty to the H_2O_2 toxicological endpoints being used for PFA.

As indicated in Table C1, PFA and PAA are structurally similar and have similar (or expectedly similar) physical and chemical properties, and the Agency agrees that PFA may generally be grouped with the other peroxy compounds. However, no comparisons of their toxicological endpoints were made, due to the lack of or limited data for all the chemicals, and the relationship between the physical/chemical properties (other than bond dissociation energy) and toxicity was not sufficiently explained. For instance, PFA is a slightly stronger acid with a pK_a ranging from 7.1 to 7.77, while PAA has a pK_a of 8.20 (Table C1). Therefore, PFA is expected to have a slightly worse irritation profile than PAA, and it is unclear where the bond dissociation energy factors into this. PFA also has greater efficacy compared to PAA, and it is unclear where the bond dissociation energy factors into this and how the efficacy may tie to toxicity. From a chemistry perspective, the relationship between each relevant property, such as acidity, and toxicity/irritation potential should be further examined to provide a stronger justification for the read across of the toxicological endpoints of PFA and other peroxy compounds.

C.3 Conclusions of Grouping Analysis

Based on general similarities between PFA, PAA, and H_2O_2 in physical/chemical properties and fate characteristics provided by the available environmental fate data, the Agency agrees that PFA may generally be grouped with the peroxy compounds. PFA is expected to behave similarly to the peroxy compounds in the environment and degrade rapidly. This rapid degradation, along with the proposed indoor use pattern and high volatility, are expected to result in minimal environmental exposure to PFA from the proposed antimicrobial use of glycerol formate.

Regarding toxicity, however, there is uncertainty surrounding the toxicity of PFA from the proposed use of glycerol formate. No comparisons of the toxicological endpoints of PFA, PAA, and H_2O_2 were made, and insufficient justification was provided to relate the physical/chemical properties with the assumed toxicity. Though the registrant indicated that the (similar) oxidizing properties of PFA and PAA are the driver for irritation potential, they did not explain the significance of other physical/chemical/fate properties, such as acidity and efficacy, on toxicity/irritation potential. Given the greater reactivity and acidity of PFA compared to H_2O_2 and PAA, respectively, PFA has greater toxicity potential compared to the other peroxy compounds. To estimate inhalation exposure risk to PFA, which is the anticipated exposure pathway of concern, the Agency is applying a database uncertainty factor of 10x to account for the use of the H_2O_2 inhalation data to assess PFA.