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Toxicology Directorate

Toxicology Assessment for Strategic Environmental Research and Development Program: Per- and Polyfluoroalkyl Substances - Free Aqueous Film Forming Foams

July 2020-June 2022

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14. ABSTRACT

This report is a Toxicology Assessment for six per- and polyfluoroalkyl substances (PFAS)-free aqueous film forming foam (AFFF) products under consideration for use by the Department of Defense. This phased assessment is in active testing and therefore this report represents the status of information available as of April 2022.

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PFAS, per- and polyfluoroalkyl substances, toxicity, CD-1 mice, acute, subacute, AFFF, aqueous film forming foam

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JULY 2020-June 2022

1 EXECUTIVE SUMMARY

1.1 Overview

Research, development, testing, training, and use of substances potentially less hazardous to human health and the environment is vital to the readiness of the U.S. Army. Safeguarding the health of Soldiers, Civilians, and the environment requires an assessment of proposed alternative chemicals and products before they are fielded. Continuous assessments begun early in the research, development, test, and evaluation (RDT&E) process can save significant time and effort if replacement materials result in a decreased requirement for occupational, industrial, or environmental monitoring and clean-up. Proactive assessment of replacement materials early in the RDT&E process may help avoid the costs of clean-up and medical management of legacy compounds. Continuous toxicity assessment performed in parallel with the RDT&E process is fully described in Technical Guide (TG) 389, *Guide to Performing a Developmental Environment, Safety, and Occupational Health Evaluation (DESHE*) (APHC 2021).

Current aqueous film forming foams (AFFFs) used by the Department of Defense (DoD) contain per- and polyfluoroalkyl substances (PFAS), which are a class of chemicals that are persistent in the environment and bioaccumulate in animals. PFAS have been found globally in drinking water sources and in tissues of animals. Some PFAS are found to bioaccumulate in the liver and other tissues and are poorly excreted in humans. The extent of the toxicological impact of PFAS exposure is an active area of research, but there is growing evidence of negative health impacts across multiple biological systems.

The Fiscal Year (FY) 2020 National Defense Authorization Act (NDAA) guides the Secretary of Defense to prohibit the release of fluorinated AFFFs into the environment outside of emergency scenarios or with containment and disposal techniques in place to preclude release to the environment (NDAA 2019 Sec. 323(a), 323(b)(1-2), and 330). Further, use of fluorinated AFFFs for training activities is prohibited (NDAA 2019 Sec. 324). The FY2021 NDAA H.R.6395 (NDAA 2021) promotes research and development of alternatives to PFAS-containing AFFFs to facilitate Military Standard (MILSPEC) development and fielding of a PFAS-free AFFF (NDAA 2020 Sec. 334). It further explicitly states the requirement to explore green and sustainable chemistry and chemicals that limit harm to public health and the environment (NDAA 2020 Sec. 334(a)(2) and 334(b)(2)). Related, the Secretary of Defense is required to leverage existing research programs within the DoD (NDAA 2020 Sec. 334(b)(3)).

To support these requirements, the U.S. Army Public Health Center (APHC) Toxicology Directorate is preparing a detailed Toxicity Assessment and performing *in vitro* and *in vivo*

toxicity testing for six PFAS-free AFFFs under consideration for use by the DoD and one PFAS-containing AFFF as a comparator. Toxicity Assessments are a tool to collate existing toxicological data, identify gaps in knowledge, and provide uncertainty assessments. Herein, data from available literature and toxicity testing performed by APHC and other Strategic Environmental Research and Development Program – Environmental Security Technology Certification Program (SERDP-ESTCP) collaborators are summarized with the goal of ranking PFAS-free AFFFs from least to most likely to be toxic to human health and the environment. This Toxicity Assessment does not assess any potential hazard from combustion products that may occur when foams are applied to active fires. Decision makers can use this information alongside cost-benefit analysis to make a data-driven decision for the adoption of PFAS-free AFFF.

1.2 Purpose

The purpose of this document is to provide a technical foundation of environment, safety, and occupational health (ESOH) hazards in a context useful to decision makers when choosing fluorine-free alternatives to PFAS-containing AFFFs. This Toxicity Assessment is in support of a large SERDP-ESTCP effort to develop and field methods to detect, remediate, and replace PFAS-containing AFFFs with PFAS-free products. This document addresses the current understanding of the environmental fate and transport, persistence, bioaccumulation, toxicity and ecotoxicity of six PFAS-free AFFFs that are in the RDT&E pipeline to replace current PFAS-containing AFFFs. Moreover, FY2020 NDAA contains specific provisions to cease use of fluorine-containing AFFFs at military installations by FY24 and to matrix DoD-wide efforts to improve understanding of PFAS-free AFFFs and reduce environmental impacts of AFFFs, in general.

1.3 Conclusions

Generally, the PFAS-free AFFF products appear to have lower likelihood of environmental persistence and bioaccumulation, and have lower oral human health toxicity than products containing PFAS. In the simplest terms, the PFAS-free AFFF products can be summarized as complex soaps. Most products in their concentrated form may cause dermal and ocular irritation and may be hazards to firefighters without the use of PPE. The extension of this is that oral exposure may cause gastrointestinal distress. Notably, one product has the potential for anemia/liver injury at high concentrations (approximately 20% dilution). Aquatic toxicity may be a concern from direct or repeated environmental releases, especially for organisms that live at or near the water/air interface where foam may collect and concentrations may elevate. Indirect releases are unlikely to have lasting impacts due to the short biodegradation half-life of PFAS-free AFFF products (i.e., reduced likelihood of aquatic receptor exposure).

All of the PFAS-free AFFF products assessed contain chemicals that are notable as plausible hazards due to release uncertainties (e.g., concentration, release volume, release timing). Additionally, each product contains chemical constituents that are below the legal reporting threshold and/or are protected as proprietary business information and are therefore not disclosed in Safety Data Sheets (SDS). Due diligence should include a holistic understanding of waste disposal practices and appropriate personal protective equipment (PPE) to protect users from concentrates, foams, and dilute waste or environmental contamination. Moreover, the

relative hazards across human occupational exposure (e.g., concentrate, foam, or dilute exposures), human environmental exposure (e.g., training, emergency response, clean-up), and environmental exposure to aquatic, mammalian, and other terrestrial species should be balanced to reduce the potential hazard across all sectors.

Based on the assumed exposure to PFAS-free AFFF products at the working concentration of 3% (i.e., the formulation-level) or less, appropriate application and clean-up, use of PPE, and waste handling will mitigate most human health hazards. An important caveat is that data on reproductive and developmental hazards are still pending, aquatic toxicity is higher than mammalian toxicity, and there is still a large gap in knowledge based on deficient identification of product components by the manufacturers. Additionally, there are no data incorporated or assessed on potential combustion products of these mixtures.

1.4 Recommendations

The summary interpretation of environmental hazards for these products is that BIOEX® ECOPOL A 3%™ has the lowest overall hazard while, Naval Research Lab (NRL)-502W, National Foam 20-391, National Foam Avio®F3 Green KHC 3%, and Fomtec® Enviro USP are largely equivocal with low hazard based on equal weighting of occupational, aquatic, mammalian toxicity, and environmental persistence, bioaccumulation, fate and toxicity (PBF&T) hazards. Solberg® Re-Healing™ Foam RF3 3% ranks as the most hazardous (see Table 14, Table 16, and Figure 5). Furthermore, based on disclosed, protected information, Solberg Re-Healing should be removed from consideration due to an inability to meet the current (draft) MILSPEC for PFAS-free AFFFs (APHC 2021, Addendum 2).

A ranking of these PFAS-free AFFF products is currently considered unreliable because of unknown constituents in their formulations, their variable constituent components that may span multiple chemical classes, and the difference in hazard profiles which makes direct comparisons difficult. Moreover, product comparisons that span diverse toxicity testing paradigms (e.g., occupational, mammalian, aquatic, persistence, bioaccumulation, and toxicity (PBT)) are an appropriate way to screen and test toxicity, but are unlikely to pinpoint the mechanism of toxicity of complex mixtures.

Legacy PFAS-containing product hazard is largely attributed to issues associated with persistence and bioaccumulation. Reproductive and developmental concerns based on longer-term exposure and point of release transport remain an uncertainty. PFAS-alternatives should be further screened for environmental PBF&T, reproductive/developmental hazard, and chronic exposure hazard in continuous exposure scenarios. Best practice to manage these concerns would be to increase focus on biodegradation testing as part of a tiered-testing approach (see TG 389 (APHC 2021)) in MILSPEC requirements and incorporate full material disclosure information provided by manufacturers into waste management requirements.

2 REFERENCES

See Appendix A for the references cited in this document.

3 AUTHORITY

Funding for this work was provided under Military Interdepartmental Purchase Request W74RDV01273067. This Toxicology Assessment addresses, in part, the ESOH requirements outlined in the following Department of the Army (DA) Regulations and Directives:

- Army Regulation (AR) 200-1, Environmental Protection and Enhancement, 2007.
- AR 40-5, Preventive Medicine, 2020.
- AR 70-1, Army Acquisition Policy, 2018.
- Department of the Army Pamphlet 40-11, Army Public Health Program, 2020.
- FY20 NDAA Sections 322, 323, 330, 2020.
- FY21 NDAA Sections 334, 2021.

The sponsor is the SERDP-ESTCP. Dr. Andrea Leeson is the Deputy Director and Environmental Restoration Program Manager, SERDP-ESTCP.

4 BACKGROUND

This Toxicity Assessment compares six PFAS-free AFFFs under development as replacements for legacy, PFAS-containing AFFF. Table 1 gives the composition of each product. The goal of this Toxicology Assessment is to: 1) summarize toxicity data of PFAS-free AFFFs using peer-reviewed data, government reports, testing data from SERDP-ESTCP collaborators, and estimation techniques; 2) highlight the information gaps that remain; and 3) recommend a path forward.

Importantly, the known components of each AFFF ranges from 5% to 74.5%, indicating that up to 95% of a product has not been disclosed by the manufacturer. While a large proportion of the unknown components could potentially be comprised of water as a solvent, relative toxicity rankings require addressing this known knowledge gap through estimation techniques, uncertainty analysis, inclusion of constituent data, and phased testing.

Verbiage used throughout the document to describe the PFAS-free AFFFs as purchased and intended to be mixed with water include AFFF, product, and concentrate because the product is sold as a highly concentrated solution. Formulation, foam, and 3% solution all refer to the AFFFs in usage (i.e., applied via a foaming nozzle). This language follows guidance of SERDP-ESTCP.

Table 1. Product Components

Product and Disclosed Components	Abbreviation	CASRN	% of Product
BIOEX ECOPOL A 3%	**% known = 10-32.5015 %**		
2-(2-Butoxyethoxy)ethanol*	DGBE	112-34-5*	10-25
Alkylbetaine		not disclosed	0-2.5
Alkylsulfate		not disclosed	0-2.5
Amphoteric Surfactant		not disclosed	0-2.5
Preservative		not disclosed	0-0.0015
Fomtec Enviro USP	**% known = 12-23 %**		
Diethylene glycol monobutyl ether*	DGBE	112-34-5*	5-10
Sulfuric acid, mono-C12-14-alkyl esters, compds with triethanolamine	AS C12-14 TEA	90583-18-9	5-9
Alcohols, C12-14, ethoxylated, sulfates, sodium salts*	AES C12-14 2.5EO Na	68891-38-3*	1-3
Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides	AO C12-14	308062-28-4	<1
National Foam 20-391	**% known = 5-24 %**		
1-Propananminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-(C8-18(even numbered) acyl) derivs., hydroxides, inner salts	CAPHS C8-18	ECN: 939-455-3	4-10
2-methyl-2,4-pentanediol*	HG	107-41-5*	4-10
Sodium laureth sulphate*	AES C12-14 2.5EO Na	68891-38-3*	1-4
National Foam Avio^{F3} Green KHC 3%	**% known = 25-61 %**		
Diethylene glycol monobutyl ether*	DGBE	112-34-5*	10-30
Lauramine oxide	AO C12	1643-20-5	7-13
Sodium lauryl sulfate	AS C12 Na	151-21-3	7-13
Dimethyltetradecylamine oxide	AO C14	3332-27-2	1-5
Naval Research Lab (NRL) 502W (siloxane-based)	**% known = 33.7 %**		
Butyl Carbitol Solvent (Diethylene glycol monobutyl ether, ≥99%)*	DGBE	112-34-5*	17
Glucopon 225DK (D-Glucopyranose, oligomers, dedyl octyl glycosides, 60-100%)*	DG	68515-73-1*	10
502W Additive; (3-(Polyoxyethylene) propylheptamethyltrisiloxane, 70-90%)	PDMS EO	67674-67-3	6.7
Solberg Re-Healing Foam RF3 3%	**% known = < 74.5 %**		
1-propanaminium, 3-amino-N-(carboxymethyl) -N,N-dimethyl-,N-coc acyl derivs., hydroxides, inner salts	CAPB C12	61789-40-0	<20
2-(2-Butoxyethoxy)ethanol*	DGBE	112-34-5*	<20
Tris(2-hydroxyethyl)ammonium dodecylsulfate	AS C12 TEA	139-96-8	<20
Alpha-sulfo-omega-hydroxy-poly(oxy-1,2-ethanediyl)C9-11 alkyl ethers, sodium salts	AES C9-11 1-3EO Na	96130-61-9	<5
D-glucopyranose, oligomers, decyl octyl glycosides*	DG	68515-73-1*	<5

Product and Disclosed Components	Abbreviation	CASRN	% of Product
1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-,N-coco acyl derivs., hydroxieds, inner salts	CAPHS C12	68139-30-0	<2.5
Starch		9005-25-8	>1
Sucrose		57-50-1	>1

Legend:
ECN = European Community Number
CASRN = Chemical Abstracts Service Registry Number
Notes:
* = repeat compound
Compounds where CASRN have not been provided by the manufacturer have been given short descriptive titles.

Current DoD regulations require assessment of human health and environmental effects arising from exposure to substances in soil, surface water, ground water, and the occupational environment (APHC 2021). Assessments performed after a chemical or compound is already in use may reveal adverse environmental and human health effects that must then be addressed, often at substantial cost. A more proactive approach is to assess exposure, effects, and environmental transport of military-related compounds or substances early in the RDT&E process to avoid unnecessary costs, conserve physical resources, and sustain the health of our Forces and others potentially exposed such as military families and fenceline communities.

In support of a proactive assessment, APHC has created a phased approach to identify potential ESOH impacts as described in TG 389 (APHC 2021). This phased assessment is in active testing and therefore this Toxicity Assessment represents the status of information available as of April 2022.

5 PROBLEM STATEMENT

PFAS-free AFFFs are being developed and tested for their efficacy and potential to elicit incidental adverse effects in animals and/or humans. Given the novelty of PFAS-free AFFFs and the paucity of toxicological data on the products, this toxicity evaluation is necessary to avoid regrettable substitutions (i.e., replacing PFAS-containing AFFFs with a product similarly or more toxic). A particular challenge is the relatively short time provided in the FY20 NDAA to change from use of these PFAS-containing materials. Specifically, Section 322 of the NDAA states that the Secretary of the Navy will publish a MILSPEC for fluorine-free AFFFs by 31 January 2023 and that agents are available for use at military installations no later than 01 October 2023. Furthermore, fluorinated AFFFs may not be used at any military installation on or after 01 October 2024.

Of note, we recognize the technical and policy disconnect surrounding “fluorine-free,” “PFAS-free,” and “<1 ppb PFAS” language throughout the NDAA and draft MILSPEC. Accordingly, our assessment and recommendations are intended to capture the intent of the NDAA, which is to limit the release of PFAS through AFFF use.

There is an urgent need to find an appropriate AFFF replacement product that performs adequately to the draft MILSPEC, preferably with low probability to cause occupational or environmental hazards. Decision makers must balance the hazards and risks associated with performance and toxicity, and environmental persistence and transport parameters. This document addresses the toxicity of six candidate PFAS-free AFFFs, summarizes our knowledge of the toxicity of each mixture, and provides a relative ranking of hazard.

Table 2 illustrates the baseline toxicity information available from manufacturer-supplied SDS and the resultant data gaps for each product (and their constituents). Manufacturer-supplied SDS are a reasonable first pass assessment tool, but rarely disclose the primary data used to classify the toxicity of each component, as is evident by the amount of white (e.g., no available information) in the table.

Our methods described in Section 6 include a thorough literature review to incorporate existing experimental data into the toxicity information table initially containing SDS data only (Table 2). This document converts toxicity and hazard data into ‘stoplight’ categories to simplify interpretation and unify hazard screening at APHC with international bodies (APPENDIX B). If data are lacking, quantitative structure-activity relationship (QSAR) modeling and other estimation techniques (i.e., read-across) can provide insight about potential toxicity or hazards of constituents (see Addendum 1). Where large data gaps remain, toxicity testing may be pursued to fill those gaps. Incorporating literature review, estimation techniques, and phased toxicity testing adds incremental confidence to the findings presented in this Toxicity Assessment.

Table 2. Toxicity Information of Product Components from Manufacturer-supplied Safety Data Sheets Illustrating Knowledge Gaps Prior to This Toxicology Assessment

	CASRN	% of Product	Eye Damage/ Irritation	Skin Corrosion/ Irritation	Skin Sensitization	Specific Organ Toxicity Single	Specific Organ Toxicity	Carcinogenicity	Germ Cell Mutagenicity	ReproTox	Acute Oral Toxicity (LD_{50})	Acute dermal toxicity (LD_{50})	Acute Inhal. Toxicity	Respiratory Sensitization	Aquatic Acute	Aquatic Chronic	LC_{50} fish	EC_{50} Daphnia 48 hr	EC_{50} *Daphnia magna*, 24 hr	ErC_{50} algae	NOEC chronic fish	Mobility in Soil	Biodegradation	Bioaccumulative Potential
BIOEX ECOPOL A 3%		**10-32.5**																						
112-34-5*	DGBE	10-25																						
Alkylbetaine		0-2.5																						
Alkylsulfate		0-2.5																						
Amphoteric Surfactant		0-2.5																						
Preservative		0-0.0015																						
Fomtec Enviro USP		**12-23**																						
112-34-5*	DGBE	5-10																						
90583-18-9	AS C12-14 TEA	5-9																						
68891-38-3*	AES C12-14 2.5EO Na	1-3																						
308062-28-4	AO C12-14	<1																						
National Foam 20-391		**5-24**																						
ECN: 939-455-3	CAPHS C8-18	4-10																						
107-41-5*	HG	4-10																						
68891-38-3*	AES C12-14 2.5EO Na	1-4																						
National Foam Avio^{F3} Green KHC 3%		**25-61**																						
112-34-5*	DGBE	10-30																						
1643-20-5	AO C12	7-13																						
151-21-3	AS C12 Na	7-13																						
3332-27-2	AO C14	1-5																						
NRL 502W (Siloxane-based)		**33.7**																						
112-34-5*	DGBE	17																						
68515-73-1*	DG	10																						
67674-67-3	PDMS EO	6.7																						
Solberg Re-Healing Foam RF3 3%		**< 74.5**																						
61789-40-0	CAPB C12	<20																						
112-34-5*	DGBE	<20																						
139-96-8	AS C12 TEA	<20																						
96130-61-9	AES C9-11 1-3EO Na	<5																						
68515-73-1*	DG	<5																						
68139-30-0	CAPHS C12	<2.5																						
9005-25-8		>1																						
57-50-1		>1																						

Legend: * = repeat compound; EC = European Community; LD_{50} = concentration resulting in 50% lethality to a population of test animals; EC_{50} = concentration resulting in 50% effect on a population of test animals; ErC_{50} = concentration resulting in 50% reduction in growth rate within 72 hours of exposure; NOEC = no observed effect concentration Notes: grey = product; white = no information; green = no toxicity; yellow = low toxicity; orange = mid toxicity; red = high toxicity; violet = very high toxicity; based on Globally Harmonized System (GHS) system of classification for each toxicity endpoint (see Appendix B for full details)

6 METHODS

6.1 Document Development

Briefly, this document follows the data requirements, format, and procedures found in TG 389 (APHC 2021). This document summarizes the hazards and general toxicity of six PFAS-free AFFFs. APPENDIX B contains details of Globally Harmonized System (GHS) hazard categorization (see related in Table 3). Addendum 1 contains summarized physicochemical parameters, toxicity data used throughout the assessment, and toxicity profiles of individual constituents identified and interpreted on an individual basis. Addendum 2 contains confidential business information (CBI) supplied to APHC by manufacturers or SERDP-ESTCP collaborators (i.e., preliminary or pre-publication data) that has been reviewed and considered in the final discussion and recommendations.

The methodology of constituent data acquisition and categorization is included here for clarity and to describe the source of data used in cases where product-level data were not identified (e.g., a “reverse” data gap, since the primary focus of this document is the whole products) and constituent-level data were used in place during the initial implementation of this Toxicity Assessment. Product-level data were interpreted through the same categorization scheme as the constituent-level data. Toxicity data provided by SDS, collected at APHC, or shared by SERDP-ESTCP collaborators are intended to inform likely exposure scenarios (e.g., concentrations used during foam application). Moreover, there are not adequate data to perform a full mechanistic assessment on these AFFF replacements.

6.2 Constituent Data Acquisition and Categorization

To determine the human health and environmental impact of compounds used in PFAS-free AFFFs, it is necessary to identify each compound correctly and its physical, chemical, and toxicological properties. The Chemical Abstracts Service registry number (CASRN) is the primary way to identify each compound in this program (see Table 1). While all compounds do not necessarily have a single CASRN, this number reduces ambiguity in accessing information for chemical substances. The CASRN is readily used as a keyword for searching online databases and is often cross-referenced with both systematic and trivial (i.e., “common” or non-systematic) names for chemical substances. In some cases, synonyms and trade names are also used to identify structures.

For compounds that do not have a CASRN, the simplified molecular-input line-entry system (SMILES) is generated within Estimation Programs Interface Suite (EPI Suite™) (U.S. Environmental Protection Agency (EPA) 2013) and searched in the PubChem database (Kim et al. 2019) to identify compounds that may have been submitted (e.g., for patent purposes) but are not in commerce.

Basic physical and chemical properties are usually determined by consulting curated databases (e.g., PubChem or European Chemical Agency (ECHA)), but when physical and chemical properties are unavailable, predictions based on chemical structure and/or SMILES are generated within EPI Suite.

The properties necessary to assess fate and transport in the environment include:

- Molecular weight (MW in grams (g) per mol; g/mol).
- Boiling point (bp) in degrees Celsius (°C).
- Octanol-water partition coefficient (log K_{OW}).
- Organic carbon partition coefficient (log K_{OC}).
- Water solubility (milligrams (mg) or milliliters (mL) per liter (L) e.g., mg/L or mL/L).
- Henry's Law constant (K_H).
- Vapor pressure (vp) in millimeters (mm) of mercury (Hg) – mmHg.

Toxicological information needed to estimate potential human health risks include reported toxicity effects of oral, inhalation, dermal, and ocular exposures; potential for developmental and reproductive toxicity, mutagenesis and carcinogenesis; and mode(s) and mechanisms of toxicity. Values reported herein include:

- Lethal dose 50% (LD_{50}; reported in milligrams (mg) per kilogram (kg) i.e., mg/kg);
- No observed adverse effect level (or concentration) (NOAEL/C) and lowest observed adverse effect level (or concentration) (LOAEL/C) reported in mg/kg per day (mg/kg-d) or mg/liter per day (mg/L-d);
- 50% effect concentration (EC_{50}) or lethal concentration 50% (LC_{50}) typically reported as mass (g or mg) per cubic meter (m^3) or mg/L; and
- Water quality values (reported in micrograms/liter (µ/L) or parts per million (ppm)).

Toxicological information was derived directly from primary sources whenever possible. Sources used for this Toxicity Assessment include National Library of Medicine databases (PubChem, PubMed, and NCBI); the EPA's databases and tools (CompTox dashboard, ECOTOX Knowledgebase, ChemView); the U.S. Department of Health and Human Services' Agency for Toxic Substances and Disease Registry (ATSDR); Google Scholar; Consumer Product Information Database (CPID); Defense Technical Information Center (DTIC®); ECHA; and publications from the National Institute for Occupational Safety and Health (NIOSH), World Health Organization (WHO), and International Agency for Research on Cancer (IARC). Commercial suppliers may provide results of in-house research that do not appear in the open literature.

Persistence, bioaccumulation, human health toxicity, and ecotoxicity were assigned to general categories of hazard (i.e., low, moderate, or high) using criteria modified from Howe et al. (2007) and described in TG 389 (APHC 2021). Table 3 describes the criteria used in the categorization, though the relative proportions of each substance were also factored into the final assessment. Where applicable, hazard is also characterized using the GHS Classification and Labeling of Chemicals (United Nations Economic Commission for Europe (UNECE) 2017) (Tables B-1 – B-4 and B-8, APPENDIX B)). In some cases, toxicity values for substances are less than category 4, and do not meet the criteria for category 5; such compounds are not classified in the GHS.

If no experimental data were located in the literature, physicochemical parameters or toxicity values are predicted using QSAR software, where possible. Modeling packages include EPA's EPI Suite 4.0 (EPA 2013), EPA's ECOSAR™ (EPA 2012), and TOPKAT (BIOVIA 2015).

Additionally, a read-across predictive approach was used for sister chemicals that belong to generalizable classes (e.g., surfactants). This provides the capability to assign physicochemical parameters or toxicity estimates for products with, for example, a common head group, but variable carbon chain lengths, that may not have adequate supporting toxicological data.

Table 3. Categorization Criteria Used in the Development of Environmental Safety and Occupational Health Severity

	Low	Moderate	High	Unknown
PERSISTENCE	Readily biodegrades water <28 days soil <28 days	Degradation ½ life: water <40 days soil <120 days	Degradation ½ life: water >40 days soil >120 days	Data are unavailable, insufficient, or unreliable
TRANSPORT	Water sol. < 10 mg/L log K_{OC} > 2.0	Water sol. 10-1,000 mg/L log K_{OC} 1.0-2.0	Water sol. > 1,000 mg/L log K_{OC} <1.0	
BIO-ACCUMULATION	log K_{OW} <3.0	log K_{OW} 3.0-4.5	log K_{OW} >4.5	
TOXICITY	No evidence of carcinogenicity/ mutagenicity (IARC group 3 & 4); Subchronic LOAEL > 200 mg/kg-d	Mixed evidence for carcinogenicity/ mutagenicity (IARC group 2B); Subchronic LOAEL 5-200 mg/kg-d	Positive corroborative evidence for carcinogenicity/mutagenicity (IARC group 1 & 2A); Subchronic LOAEL < 5 mg/kg-d	
ECOTOXICITY	Acute LC_{50} >1 mg/L or LD_{50} 1500 mg/kg; Subchronic EC_{50} >100 µg/L or LOAEL >100 mg/kg-d	Acute LC_{50} 1-0.1 mg/L or LD_{50} 1,500-150 mg/kg; Subchronic EC_{50} 100-10 µg/L or LOAEL 100-10 mg/kg-d	Acute LC_{50} <100 µg/L or LD_{50} <150 mg/kg; Subchronic EC_{50} <10 µg/L or LOAEL <10 mg/kg-d	

Legend:
sol. = solubility
mg/L = milligrams per liter
Koc = organic carbon partition coefficient
Kow = octanol-water partition coefficient
IARC = International Agency for Research on Cancer
LOAEL = lowest-observed adverse effect level
mg/kg-d = milligrams per kilogram per day
LC_{50} = concentration expected to result in 50% lethality to a population of test animals
LD_{50} = dose expected to result in 50% lethality to a population of test animals
EC_{50} = concentration expected to result in 50% effect to a population of test animals
µg/L = micrograms per liter
Note:
(Modified from Howe et al. 2007)

6.3 Constituent-level and Product-level Uncertainty Analysis

To characterize the uncertainty in these data, we developed a product-level data assurance score. There are four main sources of uncertainty identified specific to this assessment:

(i) Identification rate – the ability to identify constituents within products sufficient to search for toxicity/hazard data,

(ii) Completeness rate – searches resulting in no data leading to “no data (ND)” categorizations,

(iii) Experimental data rate – accepting predicted toxicity/hazard endpoints where experimental data are lacking, and

(iv) Percent disclosed – the percent of constituents disclosed in manufacturer-supplied SDS for products.

The ability to identify constituents within products (i) is predicated on inclusion of a CASRN on the SDS, but using read-across methods can mitigate some uncertainty when no CASRN is provided (paragraph 7.2). In some cases with high quality identification information (such as a CASRN), no data were recovered after an extensive search (ii). QSAR or read-across predictions address many of these true data gaps, but there were cases where this was not possible and “ND” categorizations are listed for certain endpoints and result in a completeness rate < 1, which indicates that there were no data or extrapolation methods available to close the data gap. The proportion of endpoint categories derived solely from experimental data is the data quality score (iii), which aims to capture the relative proportion of measured vs. predicted data in our compiled dataset. The sum of the three proportions (i-iii; identification rate, completeness rate, and experimental data rate) is the data quality score. The data quality score range is 0 to 3 with a high score of 3 indicating complete identification data, complete endpoints represented in a literature search, and all endpoints are derived using experimental data.

The last source of uncertainty – the dominant form of uncertainty impacting the assessment of toxicity/hazard of these products – is percent disclosure. In the U.S., manufactures are not required by law to disclose 100% of their product, but rather only hazardous chemicals present at ≥ 1% or hazardous chemicals present at < 1% (< 0.1% for carcinogens) with evidence that release from the mixture may exceed an established Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit or American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value, or could present a health risk to employees (29 CFR 1910.1200(g)(2)(i)(C)(2)). SDS disclosure of less than 100% of constituents in a product is not always a constraint, but in this specific case, proportions disclosed in SDSs range from 5% to 75% indicating a highly variable level of uncertainty that needs to be captured. Accordingly, the final metric produced in the uncertainty analysis—the data assurance score—is the data quality score weighted by the maximum percent of the product disclosed in the SDS (iv). Accordingly, the data assurance score ranges from 0 to 3 with a 3 representing maximum assurance in the combined dataset per product.

The data assurance scores presented in this document are based on product-level data from testing (at APHC and by SERDP-ESTCP collaborators) and from SDS sources. Accordingly, the data quality score is calculated directly at the product-level and this single value is then weighted by the percent product disclosed in SDS. Constituent-level data is presented in Addendum 1 with the constituent toxicity profiles and other constituent-level data.

The data assurance score is intended to be independent of, but used alongside, relative toxicity rankings to provide decision-makers with a semi-quantifiable metric of assurance in the dataset used to create the rankings. It is intended to aid in downselection of products that have similar toxicity rankings—e.g., a product with a high hazard ranking but low data assurance score should not be excluded from future toxicity tests, while a high hazard ranking product with high data assurance score could be excluded from future tests or even consideration as a replacement product.

6.4 *In vitro* Testing of Products

In vitro toxicity testing of products in Table 1 was performed by APHC Toxicology Directorate to assess skin irritation hazard, potential for DNA damage through mutation, and toxicity estimates to aquatic/ecological receptors. Skin irritation is assessed using reconstituted human skin epidermis at a single (relevant) concentration of 3% weight per volume (w/v) to mimic field usage conditions and likely human/occupational exposures. DNA damage (i.e., mutagenicity) was assessed in a modified Ames assay with *Salmonella typhimurium* TA100 with and without rat liver metabolic S9. Aquatic toxicity estimates were determined in a Microtox assay with *Aliivibrio fischeri* luminescent bacteria, which is considered predictive of EC_{50} estimates in the standard ecological receptor *Pimphales promelas.* These *in vitro* assays are intended to serve as screening tools to reduce time to final hazard interpretation and alternative down-selection.

6.5 *In vivo* Testing of Products

In vivo toxicity testing was performed by APHC Toxicology Directorate to assess acute and short-term repeat dose toxicity using CD-1™ mice (Charles River Lab). In the acute (limit) test, animals received a single dose via oral gavage of products listed in Table 1 at 2,000 mg/kg followed by 14 days of observation. Animals were weighed at least weekly and organ masses were collected at the conclusion of the study to assess potential bioenergetic or gross organ effects. In the subacute test, mice were dosed via oral gavage at 0, 125, 250, 500, 1,000, or 2,000 mg/kg-d (for each product) for 28 days. Mice were observed daily for clinical effects and weighed at least weekly. Samples were collected for MNA analysis (males only; 500, 1,000, and 2,000 mg/kg-d groups), hematology, clinical chemistry, and thyroid hormone analysis. Organ tissues were weighed and prepared for histopathological analysis. All endpoints beyond weekly weights were collected at necropsy on day 29. Animal testing occurred under Institutional Animal Care and Use Committee (IACUC) Protocol # 26-20-11-01, which was reviewed and approved by the APHC IACUC.

6.6 Collaborator Data

Testing data from SERDP-ESTCP collaborators have been incorporated into this report, as they were specifically focused on testing the same suite of AFFFs. These data were largely focused on aquatic plants, invertebrates, and fish, and have been aligned and incorporated with the most relevant endpoints addressed in this assessment. Data were retrieved during the 2021 SERDP-ESTCP Winter Symposium from posters and presentations of collaborators (Hoverman 2021, Kuperman 2021, Suski 2021, Wirth 2021, Wu 2021) and through direct request per SERDP-ESTCP technical committee feedback (anticipated initial data compilation by June 2022 and on-going thereafter). Data were either presented directly in tables by original authors or were extracted from figures and then aligned with the appropriate GHS hazard categorization as is used throughout this assessment. Accordingly, highly precise values were not necessarily attained in the initial data scrape, and these data should be considered preliminary until final publication by the originating authors or their institutions.

6.7 Relative Toxicity Ranking

There is risk in ranking products by toxicity without complete identification of constituents or product-level testing data due to potentially impactful uncertainty. However, inclusion of data from non-experiment sources (paragraph 6.2) has filled some data gaps and an uncertainty analysis (paragraph 6.3) puts uncertainty into context. Using the most up-to-date testing data from APHC and SERDP-ESTCP collaborators and data on the individual constituents from literature review, modeling, and read-cross to cover experimental data gaps, we have performed product-level toxicity ranking. The data used for ranking are visually summarized in Table 14, with circles representing summarized constituent data.

To perform a quantitative ranking, GHS and Howe et al. (2007) categories were used to scale data for each endpoint and each product. Accordingly, a higher score is better (i.e., less hazard or toxicity). Scores range from 1 to 5 to capture the maximal range of GHS and Howe et al. (2007) categories. In cases of mismatches between SDS, literature, or testing data, an average was used (e.g., category 4 and category 5 mismatch would be quantified as score of 4.5). This approach differs from using raw data and partially insulates against impacts of uncertainty around predicted endpoint values or values derived from a read-across approach. In essence, given the uncertainty around a prediction quality, the one order of magnitude width between GHS/Howe categories captures this uncertainty.

The software ToxPi v2.3 (ToxPi 2022, Marvel et al. 2018, Reif et al. 2010) was used to visualize ranking of products and calculate scores. ToxPi visuals are radar plots that are construed to look like pie charts. Each slice of the pie (vertices of the radar plot) represents a specific category of concern—in this case, each category represents multiple endpoints and describes general hazard areas (Table 4). The angle of the slice indicates the weight of that slice in the overall score—in this case, angles are equal indicating each categorical slice is equally weighted, although there are varying quantities of endpoint data compiled and scaled within each category or slice. The length of the slice indicates the score of that product in that category—in this case, longer is better (higher score indicates less hazard, analogous to LD_{50}) or “more pie is better than less pie.” Lastly, a final score is calculated for each product based on the mean of the category scores.

Table 4. Endpoints from 'Stoplight' Interpretive Matrix that Make Up the Four ToxPi Slices

Slice	Percent	Color	Endpoints
Aquatic and Ecotoxicity	25	Green	Algae EC50, Daphnia EC_{50}, Fish LC_{50}, Acute Aquatic, Chronic Aquatic, Fish NOEC
Occupational Health	25	Purple	Dermal Irritation, Dermal Sensitization, Ocular Irritation, Carcinogenicity, Mutagenicity
Mammalian	25	Red	Dermal LD_{50}, Inhalation, Oral LD_{50}, DART LOAEL, Oral LOAEL
Persistence, Bioaccumulation, Fate & Transport	25	Blue	Bioaccumulation, Biodegradation, Soil Mobility

Legend:
EC_{50} = concentration expected to result in 50% effect to a population of test animals
LC_{50} = concentration expected to result in 50% lethality to a population of test animals
NOEC = no observed effect concentration
LD_{50} = dose expected to result in 50% lethality to a population of test animal
DART = developmental and reproductive toxicity
LOAEL = lowest-observed adverse effect level

ToxPi's methodology of relative scoring is based on summing each endpoint value within a category for each product. These sums are then scaled to between 0 and 1 representing the most hazardous (0) to the least hazardous (1). This essentially mimics a relative potency amongst the products by category (this is different than relative potency by constituent/chemical common to other mixture toxicity methods). If data are not present, the "ND" is converted to a 0—this scenario does not exist in this dataset, but it is worth noting. From there, the mean of each categories' score (length of slices) is used to represent the products.

Of note, the datasets used to validate ToxPi (Marvel et al. 2018, Reif et al. 2010) are in the hundreds of chemicals and endpoints. The dataset used for this Toxicity Assessment uses 0 to 1 scaling and averaged values to represent seven products across seven categories of 19 endpoints. As a smaller sample set, the resulting ToxPi rankings should be interpreted with some caution. These quantitative techniques for relative ranking of the products also do not capture the chemical complexity/uncertainty of the products themselves.

Finally, the data assurance scores were kept independent from the ToxPi calculations to retain a two-factor interpretation of ranking.

6.8 Confidential Business Information and Limited Distribution Data

Confidential business information (CBI) received by APHC Toxicology Directorate are described in Addendum 2 to this report. This Addendum includes information from AFFF manufacturers, SERDP-ESTCP collaborators, and refined recommendations based on disclosed CBI. Key takeaways will be provided in the discussion, conclusions, and recommendations sections of this report, but specific details will remain confidential.

7 RESULTS

7.1 Physical and Chemical Properties

Table 2 in Addendum 1 summarizes physical and chemical properties. When data were not found, "ND" is indicated. In some cases, the property named is not applicable (“NA”) to the substance being described. For example, if the compound is a nonvolatile solid or an inorganic salt, vapor pressure, K_{OW}, K_{OC}, and K_H are typically negligible. In cases where an estimation is provided (through direct modeling like QSARs or read-across from similar compounds), the source is provided in Addendum 1.

7.2 Individual Constituent Class Summaries

The six potential replacement PFAS-free AFFFs assessed in this document are comprised of 21 unique compounds, which fall into five general classifications: amphiphilic solvents, amphoteric surfactants, anionic surfactants, nonionic surfactants, and carbohydrates. There is also one unidentified compound that remains unclassified. These 21 constituents are strictly sourced from SDS provided by manufacturer and, after including the assumption that the majority of non-disclosed volume is water, likely do not completely describe the full makeup of the AFFFs.

It is extremely informative to classify the components found in these AFFFs by class because many of the components are present as mixtures. Mixtures of compounds with similar head structures, but with varying carbon chain lengths may have less safety information relative to their individual carbon chain length cousins. Each component has been identified as a class and subclass (see Table 5). Tables 3 and 4 in Addendum 1 summarize the mammalian toxicity and ecotoxicity data, respectively, identified during initial constituent-level literature review and search. Further data on individual constituents used in summary and interpretive results, uncertainty analysis, discussion, and conclusions are available in Addendum 1.

Table 5. Class-based Identification of Individual Constituents

Manufacturer	Product	Constituent Class	Subclass	Min %	Max %	CAS
BIOEX	ECOPOL A 3% FFF	Amphiphilic Solvent	Glycol Ether	10	25	112-34-5
		Amphoteric Surfactant	Alkylbetaine	0	2.5	Unknown
		Amphoteric Surfactant	Amphoteric Surfactant	0	2.5	Unknown
		Anionic Surfactant	Alkyl Sulfate	0	2.5	Unknown
		Unknown	Unknown	0	0.0015	Unknown
Fomtec	Enviro USP	Amphoteric Surfactant	Amine Oxides	NA	1	308062-28-4
		Amphiphilic Solvent	Glycol Ether	5	10	112-34-5
		Amphoteric Surfactant	Organosubstituted Sulfates	5	9	90583-18-9
		Anionic Surfactant	Alkylethoxy Sulfates	1	3	68891-38-3
National Foam	20-391	Amphiphilic Solvent	Diol	4	10	107-41-5
		Amphoteric Surfactant	Alkyl Hydroxysultaines	4	10	ECN:939-455-3
		Anionic Surfactant	Alkylethoxy Sulfate	1	4	68891-38-3
National Foam	Avio^{F3} Green KHC 3%	Amphiphilic Solvent	Glycol Ether	10	30	112-34-5
		Amphoteric Surfactant	Amine Oxide	7	13	1643-20-5
		Anionic Surfactant	Alkyl Sulfate	7	13	151-21-3
		Amphoteric Surfactant	Amine Oxide	1	5	3332-27-2
NRL	502W (Siloxane-based)	Amphiphilic Solvent	Glycol Ether	NA	17	112-34-5
		Nonionic Surfactant	Alkyl Glucosides	NA	10	68515-73-1
		Amphoteric Surfactant	Ethoxylated Siloxane Surfactant	NA	6.7	67674-67-3
Solberg	Re-Healing Foam RF3 3%	Amphiphilic Solvent	Glycol Ether	NA	20	112-34-5
		Amphoteric Surfactant	Amidopropyl Betaines	NA	20	61789-40-0
		Amphoteric Surfactant	Organosubstituted Sulfate	NA	20	139-96-8
		Amphoteric Surfactant	Alkylethoxy Sulfates	NA	5	96130-61-9
		Nonionic Surfactant	Alkyl Glucosides	NA	5	68515-73-1
		Amphoteric Surfactant	Alkyl Hydroxysultaines	NA	2.5	68139-30-0
		Carbohydrate	Carbohydrate	1	NA	57-50-1
		Carbohydrate	Carbohydrate	1	NA	9005-25-8

Legend:
NA = not applicable

7.2.1 Amphiphilic Solvents

Amphiphilic solvents are characterized by their ability to partition immiscible aqueous and organic solvents. In practice, this characteristic is often applied in solutions requiring interaction between aqueous and organic solvents. Related to these AFFFs, their purpose is largely to stabilize the surfactant constituents at relatively high concentration (via micelle formation) in aqueous emulsions and to stabilize foaming during application.

Amphiphilic solvents comprise 10 – 30% of each product (20.4% on average). In all products, solvents, in general, comprise the single largest SDS disclosed proportional constituent or share the largest contribution to sum with a surfactant and, thus, are primary ingredients. Specifically, every product uses one of two solvents. HG [CASRN 107-41-5] is used in only one of the six replacements. DGBE [CASRN 112-34-5] is used in five of the PFAS-free AFFFs.

Given the disclosed information that the remainder of NRL 502W not reported on SDS is water, it is reasonable to assume that other products contain water, which would also function as a primary or secondary solvent in these products, albeit aqueous.

Disclosed, protected information (Addendum 2) indicates that products with surfactant hydrocarbons, amphiphilic solvents, and phase-transfer catalysts may result in highly variable molecule size and shape but all with similar structure, purpose, and likely similar hazards.

7.2.2 Amphoteric Surfactants

Amphoteric surfactants are characterized by their pH-dependent ionic state—cationic at low pH, nonionic at high pH, and zwitterionic at moderate pH values. Important to this assessment, amine oxides, for instance, cover the range of cationic, zwitterionic, and nonionic at environmentally relevant pH (5-9) (Belanger et al. 2016). Relevant to the constituents in these products, this characteristic arises from a quaternary nitrogen atom (cationic) and an oxygen atom (anion) or a carboxylate group (anion) with a long hydrocarbon chain opposite the polar group. The overall structure of head and tail groups is similar to other surfactants, but the amphoteric polar group allows for variable and flexible applications as foam boosters, stabilizers, detergents, and antimicrobial agents.

Amphoteric surfactants are the most common SDS-reported individual surfactant constituents in these AFFFs. At least one type of amphoteric surfactant appears in all six replacements (mean = 1.8 amphoteric surfactants per product), and one product (Solberg Re-Healing Foam RF3 3%) contains four different amphoteric surfactants. SDS data suggest that amphoteric surfactants make up a wide range of products—lowest maximum proportion reported is 1% while the highest maximum is 20% with a mean of 8.6%. Importantly, there are no repeat CASRN, and 6 of 11 reported constituents are mixtures of varying hydrocarbon chain lengths on common polar moieties. The remainder are either confirmed as high purity monomers or of unknown purity based on the data available.

7.2.3 Anionic Surfactants

Anionic surfactants are characterized by their negatively charged hydrophilic head group bonded to a hydrocarbon chain. Often these surfactants are produced as salts with cationic metals such as sodium. The most common head groups are sulfates, which is also observed in the products' anionic surfactant constituents. The hydrophobic tails of anionic surfactants vary widely based on their source material, but one of the more common deviations from simple carbon chains is an ethoxylated carbon chain. Accordingly, these surfactants are identified by their cationic metal, head group, carbon chain length, and other features such as number of ethoxy groups. Specific to these products, sodium sulfates are the most common ion pairing and carbon chains are either 12 or a mixture of 12-14 carbons. Beyond slight differences in carbon chain lengths, there is also one ethoxylated anionic surfactant.

Anionic surfactant is the second most common SDS-reported surfactant in these AFFFs. Four different products contain single anionic surfactants, and one of them is repeated (CAS 68891-38-3, 12-14 carbon ethoxylated sodium salt). The maximum proportion of anionic surfactant in a product is 13%, minimum is 2.5%, with a mean of 5.6%. Of note, one of these anionic surfactants is an alkyl sulfate that is not identified by CASRN—it is likely, given the common structures and manufacturing processes, that this alkyl sulfate is similar to AS C12 Na or could be a mixture of varying carbon chain length sulfates.

7.2.4 Carbohydrates

Carbohydrates are a generic class of chemical characterized by carbon and hydrogen bonds. They are ubiquitous in natural and synthetic chemical processes and exhibit a wide range of functional characteristics. Hydrocarbon chains are important fragments of surfactants and even amphiphilic solvents, which are relevant constituents in these AFFFs. Importantly, their production is plant-based and their environmental half-life is short.

7.2.5 Nonionic Surfactants

Nonionic surfactants are characterized by the lack of a charged hydrophilic head group. They retain the carbon chain tail of other surfactants, but have covalently bonded, oxygen-containing head groups that add hydrophilic properties. While there are a large variety of nonionic surfactants on the market, those relevant to these AFFFs are derived from plant fatty acids and glucose (alkyl glucosides). Important to the identification of these constituents, nomenclature and structures identified by CASRN and general class indicate that these surfactants may be produced as monomers or as mixtures with varying carbon chain length.

Nonionic surfactants are the least common surfactant in these AFFFs—a single CASRN is common across two of the products. In the two products, an alkyl glucoside is maximally present at 5 or 10% of the product.

7.3 Individual Constituent 'Stoplight' Interpretive Matrices

Summaries of individual constituent hazard data are presented in Table 6 and Table 7. Data were interpreted through GHS categories and categories developed by Howe et al. (2007) with purple/red being high hazard, orange/yellow being moderate hazard, and green being low or uncategorized hazard. Blue indicates the ability to search for an endpoint for the given constituent, but no endpoint data were recovered. Gray boxes are visual delineations for whole products unless filled which represents data for whole foam hazards (sourced from SDS). White boxes indicate that no data were presented in the SDS and there was a lack of information available to complete the specific endpoint reviews.

Constituent data are based on a mix of literature review, QSAR predictions, read-across, and SDS-sourced data, and is subject to all these sources' inherent variability and uncertainty. Uncertainties with constituent categorization interpretation were identified as:

(i) The ability to identify the constituent,

(ii) Detection of data specific to that constituent,

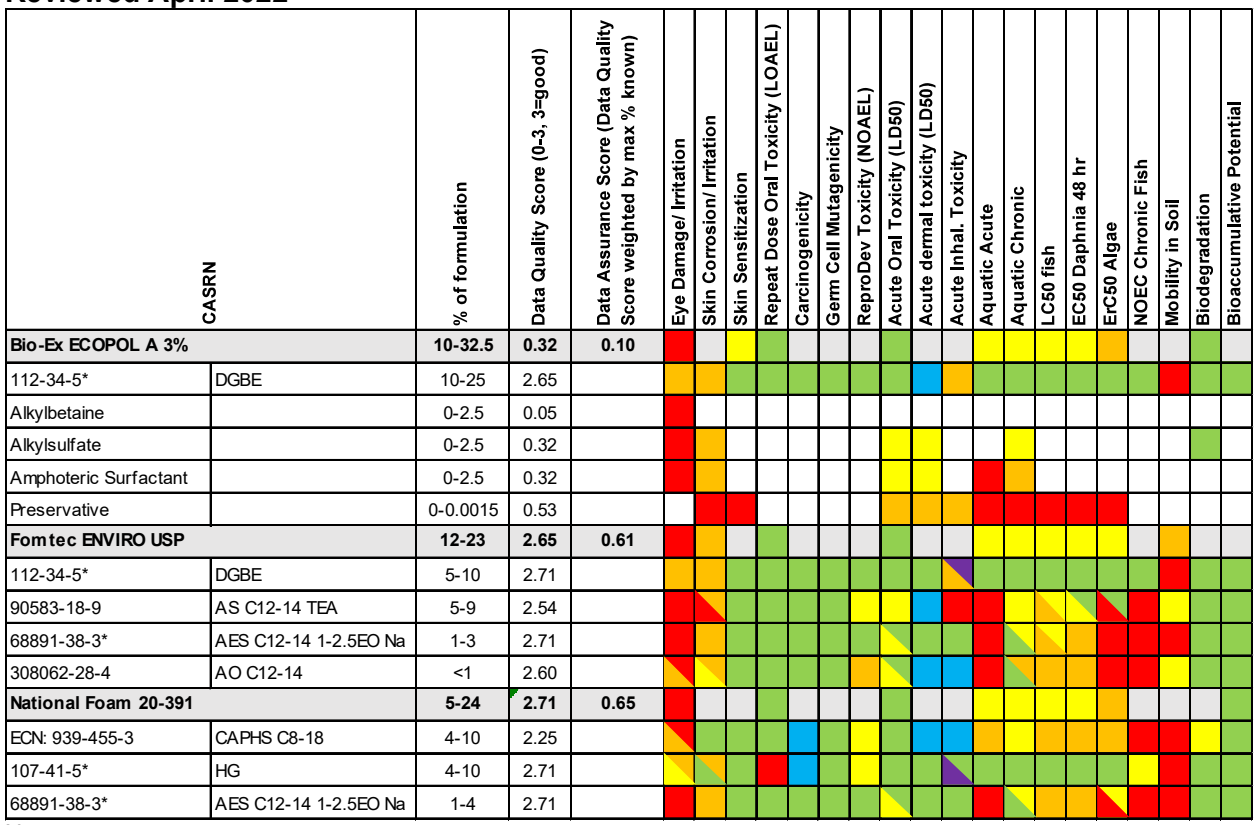
(iii) Data derived from experiments vs. predictions, and

(iv) Percent of product disclosed in SDS as described in paragraph 6.3.

See Addendum 1 for a complete description of constituent-level uncertainty analysis, individual toxicity profiles, and overall constituent-level interpretation that contribute to this summary table.

Table 6. Toxicity Information Using Manufacturer-supplied SDS, Literature Review, and QSAR Modeling for AFFF Replacement Products as Supplied by Manufacturer (Part 1), Reviewed April 2022

CASRN		% of formulation	Data Quality Score (0-3, 3=good)	Data Assurance Score (Data Quality Score weighted by max % known)	Eye Damage/ Irritation	Skin Corrosion/ Irritation	Skin Sensitization	Repeat Dose Oral Toxicity (LOAEL)	Carcinogenicity	Germ Cell Mutagenicity	ReproDev Toxicity (NOAEL)	Acute Oral Toxicity (LD50)	Acute dermal toxicity (LD50)	Acute Inhal. Toxicity	Aquatic Acute	Aquatic Chronic	LC50 fish	EC50 Daphnia 48 hr	ErC50 Algae	NOEC Chronic Fish	Mobility in Soil	Biodegradation	Bioaccumulative Potential
Bio-Ex ECOPOL A 3%		**10-32.5**	**0.32**	**0.10**																			
112-34-5*	DGBE	10-25	2.65																				
Alkylbetaine		0-2.5	0.05																				
Alkylsulfate		0-2.5	0.32																				
Amphoteric Surfactant		0-2.5	0.32																				
Preservative		0-0.0015	0.53																				
Fomtec ENVIRO USP		**12-23**	**2.65**	**0.61**																			
112-34-5*	DGBE	5-10	2.71																				
90583-18-9	AS C12-14 TEA	5-9	2.54																				
68891-38-3*	AES C12-14 1-2.5EO Na	1-3	2.71																				
308062-28-4	AO C12-14	<1	2.60																				
National Foam 20-391		**5-24**	**2.71**	**0.65**																			
ECN: 939-455-3	CAPHS C8-18	4-10	2.25																				
107-41-5*	HG	4-10	2.71																				
68891-38-3*	AES C12-14 1-2.5EO Na	1-4	2.71																				



Notes:
Gray bars are visual breaks between products; colored squares indicate data for products (from SDS, experimental, and predicted data).
For square split diagonally: lower/left triangle = data sourced from literature review; upper/right triangle = data sourced from SDS.
Blue = no data found
White = no search performed
In general, colors indicate GHS classification (see Appendix B for full details) where green = no toxicity, yellow = low toxicity, orange = moderate toxicity, red = high toxicity, violet = very high toxicity.
Biodegradation: green = readily biodegradable; yellow = not readily biodegradable.
Mobility in soil (based on log Koc and water solubility): green = low, yellow = moderate, red = high.
Bioaccumulation potential (based on log Kow and BCF value): green = low, yellow = moderate, red = high.
Repeat dose toxicity (based on Howe 2007): green = low, yellow = moderate, red = high.
ReproDev Tox: green = >1,000 mg/kg, yellow = >500 mg/kg, orange = >100 mg/kg, red = <100 mg/kg.
NOEC Chronic Fish (based on Howe 2007 using EcoTox LOAEL): green = low, yellow = moderate, red = high.

Table 7. Toxicity Information using Manufacturer-supplied SDS, Literature Review, and QSAR Modeling for AFFF Replacement Products as Supplied by Manufacturer (Part 2), Reviewed April 2022

CASRN		% of formulation	Data Quality Score (0-3, 3=good)	Data Assurance Score (Data Quality Score weighted by max % known)	Eye Damage/ Irritation	Skin Corrosion/ Irritation	Skin Sensitization	Repeat Dose Oral Toxicity (LOAEL)	Carcinogenicity	Germ Cell Mutagenicity	ReproDev Toxicity (NOAEL)	Acute Oral Toxicity (LD50)	Acute dermal toxicity (LD50)	Acute Inhal. Toxicity	Aquatic Acute	Aquatic Chronic	LC50 fish	EC50 Daphnia 48 hr	ErC50 Algae	NOEC Chronic Fish	Mobility in Soil	Biodegradation	Bioaccumulative Potential
National Foam Avio^{F3} Green KHC 3%		**25-61**	**2.74**	**1.67**																			
112-34-5*	DGBE	10-30	2.71																				
1643-20-5	AO C12	7-13	2.77																				
151-21-3	AES C12 1EO Na	7-13	2.95																				
3332-27-2	AO C14	1-5	2.65																				
NRL 502W		**33.7**	**2.65**	**0.89**																			
112-34-5*	DGBE	17	2.65																				
68515-73-1*	DG	10	2.69																				
67674-67-3	PDMS EO	6.7	1.89																				
Solberg Re-Healing Foam RF3 3%		**<74.5**	**2.49**	**1.85**																			
61789-40-0	CAPB C12	<20	2.43																				
112-34-5*	DGBE	<20	2.71																				
139-96-8	AS C12 TEA	<20	2.18																				
96130-61-9	AES C9-11 1-3EO Na	<5	1.89																				
68515-73-1*	DG	<5	2.61																				
68139-30-0	CAPHS C12	<2.5	2.54																				
9005-25-8		>1																					
57-50-1		>1																					

Notes:
Gray bars are visual breaks between products; colored squares indicate data for products (from SDS, experimental, and predicted data).
For square split diagonally: lower/left triangle = data sourced from literature review; upper/right triangle = data sourced from SDS.
Blue = no data found
White = no search performed
In general, colors indicate GHS classification (see Appendix B for full details) where green = no toxicity, yellow = low toxicity, orange = moderate toxicity, red = high toxicity, violet = very high toxicity.
Biodegradation: green = readily biodegradable; yellow = not readily biodegradable.
Mobility in soil (based on log Koc and water solubility): green = low, yellow = moderate, red = high.
Bioaccumulation potential (based on log Kow and BCF value): green = low, yellow = moderate, red = high.
Repeat dose toxicity (based on Howe 2007): green = low, yellow = moderate, red = high.
ReproDev Tox: green = >1,000 mg/kg, yellow = >500 mg/kg, orange = >100 mg/kg, red = <100 mg/kg.
NOEC Chronic Fish (based on Howe 2007 using EcoTox LOAEL): green = low, yellow = moderate, red = high.

7.4 Testing Data with Products

Given the large amount of potential and observed uncertainty around the makeup of these AFFFs and the individual constituents reported as mixtures, toxicity testing was pursued to clarify the toxicity and hazard of the products to support downstream interpretation and relative ranking.

In-house *in vitro* toxicity testing of the six products using *A. fischeri* luminescent bacteria in a Microtox acute aquatic toxicity assay found that all products were classified as acutely toxic to varying effect levels except NRL 502W (see Table 8).

Table 8. Aquatic Toxicity Via Microtox Assay

Compound	Microtox EC_{50} (mg/L) [95% CI]			Hazard Categories (EPA, 2017)	Hazard Classes (OECD, 2001)	Acute Aquatic Toxicity (GHS, 2005)
	5 min	15 min[a]	30 min			
BIOEX ECOPOL A 3%	10.67 [7.08-4.34]	7.21 [4.34-11.98]	5.91 [3.52-9.87]	Moderately Toxic	Acute Tox. II	Acute Cat. 2
Fomtec Enviro USP	6.39 [2.85-14.31]	3.75 [1.3-10.72]	2.86 [0.85-9.7]	Moderately Toxic	Acute Tox. II	Acute Cat. 2
National Foam 20-391	43.9 [24.78-77.77]	29.95 [19.98-44.91]	22.57 [14.72-34.61]	Slightly Toxic	Acute Tox. III	Acute Cat. 3
National Foam Avio^{F3} Green KHC 3%	1.3 [0.31-5.59]	0.60 [0.34-10.59]	0.43 0.012-15.3]	Highly Toxic	Acute Tox. I	Acute Cat. 1
NRL 502W	213.8 [160.7-284.4]	231 [171.6-310.9]	244.8 [183-327.4]	Practically Nontoxic	---	---
Solberg Re-Healing Foam RF3 3%	23.09 [12.79-41.7]	14.25 [7.69-26.39]	10.45 [4.99-21.9]	Slightly Toxic	Acute Tox. III	Acute Cat. 3

Legend:
Mg/L = milligrams per liter
CI = confidence interval

In-house skin irritation testing of the six products as a 3% w/v solution in water using a reconstructed human epidermis *in vitro* irritation assay found that all products were classified as non-irritants (see Table 9). Additionally, screening for mutagenicity using a modified Ames assay with *S. typhimurium* TA100 was negative with or without rat liver metabolic S9 activation for all products below the level of cytotoxicity.

Table 9. In-house Skin Irritation Testing of AFFF 3% (Concentration Used during Foam Application)

Compound	Mean Viability (% of control) ± SD	Classification
BIOEX ECOPOL A 3%	105.5 ± 5.62	Non-irritant
Fomtec Enviro USP	106.4 ± 8.69	Non-irritant
National Foam 20-391	80.4 ± 27.05[a]	Non-irritant
National Foam Avio^{F3} Green KHC 3%	98.9 ± 2.25	Non-irritant
NRL 502W	101.6 ± 3.24	Non-irritant
Solberg Re-Healing Foam RF3 3%	98.2 ± 2.74	Non-irritant

Legend:
SD = standard deviation
Note:
[a] One tissue had lower viability compared to the other two. No effect on hazard classification occurs in the absence of these data. Recommend re-testing; test was not qualified for acceptance.

In-house *in vivo* toxicity testing of the six products in CD-1™ mice through both acute and 28-day repeat-dose exposures found that all products fell under unclassified GHS categories (Table 10, Table 11, Table 12) or as low to moderate concern driven solely by clinical chemistry effects in NRL 502W (Table 12). Acute exposures consisted of a single oral dose of 2,000 mg/kg (approximately 20% concentrate dissolved in deionized water) and at least 14 days of observation. No lethality was observed, no impacts to bodyweight were detected, and only NRL 502W presented statistically significant organ weight effects (Table 10). Short-term repeat dose exposures consist of 28 days of repeat oral doses of products dissolved in deionized water plus a deionized water control. The test concentrations were 125, 250, 500, 1,000, and 2,000 mg/kg-d. In the short-term repeat dose tests, no lethality was observed, body weight reductions were observed in the highest treatment group in AvioGreen and ECOPOL A; and ECOPOL A and NRL 502W showed reductions in organ weights at the highest treatments (adrenal relative to brain weight in ECOPOL A and liver relative to body weight in NRL 502W (Table 11). *Ex vivo* assays of short-term repeat dose samples indicate Fomtec Enviro USP had the most impact on clinical chemistry parameters, NRL 502W showed significant hematological effects at the lowest concentration tested (125 mg/kg-d), no products resulted in positive responses in the MNA analysis, and potential thyroid hormonal modulation was observed in the highest treatment groups of AvioGreen and NRL 502W (Table 12). Clinical observation of transient bloating (deemed Flatulent Mouse Syndrome or FMS) was only identified in products containing DGBE solvent (Table 12), but there were not sufficient data to determine presence or absence of a dose-response relationship due to the low incidence of observed FMS.

In summary, the acute and short-term repeat dose testing in mice indicate that NRL 502W has the highest likelihood of hazard (greatest number of endpoints impacted) as well as the lowest concentration showing effects (125 mg/kg-d results in significant hematological impacts). AvioGreen shows similar amounts of endpoints impacted plus gross bioenergetic impacts (reduction in bodyweight).

Table 10. Summary of Limit Test Study Endpoints by Product

	Limit Test		
	LD_{50} [a]	**Bodyweight**	**Organ**
BIOEX ECOPOL A 3%	>2,000	NE	NE
Fomtec Enviro USP	>2,000	NE	NE
National Foam 20-391	>2,000	NE	NE
National Foam Avio^{F3} Green KHC 3%	>2,000	NE	NE
NRL 502W	>2,000	NE	M, Kidney, ↑
Solberg Re-Healing Foam RF3 3%	>2,000	NE	NE

Legend:
LD_{50} = dose expected to result in 50% lethality to a population of test animal
NE = no effect
M = male
Notes:
Limit test is single exposure to 2,000 mg/kg bolus followed by at least 14 days observation.
↑ = statistically significant increase
[a] Measured in mg/kg.

Table 11. Summary of Subacute Test Study Endpoints by Product

	Subacute (28d)		
	LD_{50}[a]	**Bodyweight**	**Organ**
BIOEX ECOPOL A 3%	>2,000	M, ↓	F, Adrenal, ↑
Fomtec Enviro USP	>2,000	NE	NE
National Foam 20-391	>2,000	NE	NE
National Foam Avio^{F3} Green KHC 3%	>2,000	M, ↓	NE
NRL 502W	>2,000	NE	M, Liver, ↓
Solberg Re-Healing Foam RF3 3%	>2,000	NE	NE

Legend:
LD_{50} = dose expected to result in 50% lethality to a population of test animal
NE = no effect
M = male
Notes:
Subacute is repeat exposures for 28 days to one of six concentrations (including control) with the highest at 2,000 milligrams per kilogram per day (mg/kg-d).
↑ = statistically significant increase
↓ = statistically significant decrease
[a] Measured in mg/kg-d.

Table 12. Continued Summary of Subacute Test Study Endpoints by Product

	Subacute (28d)				
	Clinical Chemistry[a,b]	**Hematology**	**MNA**	**Thyroid**	**FMS[c]**
BIOEX ECOPOL A 3%	↓(1),↑(2)	NE	NE	NE	4
Fomtec Enviro USP	↓(3),↑(3)	NE	NE	NE	1
National Foam 20-391	↓(1),↑(1)	↑(1)	NE	NE	0
National Foam Avio^{F3} Green KHC 3%	↓(3),↑(1)	↓(1),↑(2)	NE	F,T4[e],↓	8
NRL 502W	↓(1),↑(2)	↓(3)[d]	NE	F,T3[f],↑	7
Solberg Re-Healing Foam RF3 3%	↓(3),↑(1)	↓(1)	NE	NE	3

Legend:
NE = no effect
MNA = micronucleus assay
FMS = flatulent mouse syndrome (transient bloating)
Notes:
Subacute is repeat exposures for 28 days to one of six concentrations (including control) with the highest at milligrams per kilogram per day (mg/kg-d).
↑ = statistically significant increase
↓ = statistically significant decrease
[a] Count of parameters with changes.
[b] All products had decrease in total protein.
[c] Count of all observed bloating cases.
[d] Impact at 125 mg/kg-d; no NOAEL determined.
[e] Triiodothyronine
[f] Thyroxine

7.5 Product-Level Stoplight Interpretive Matrix

Constituent level stoplight interpretive matrices summarize major patterns using GHS and Howe et al. (2007) hazard categories (paragraph 7.3). The purpose is to infer hazards associated with products but acknowledge and account for data gaps and disparate data sources (e.g., primary literature, prediction, SDS). With the acquisition of testing data with whole products, a stoplight interpretative matrix has been constructed to include data specific to the whole products (Table 13). To account for additional data gaps, we selected the highest hazard GHS category constituent per product given the available data and for relevant endpoints using information consolidated from Addendum 1 (see Table 14, Table 15, and paragraph 7.3).

Table 13. Toxicity Information and Data Quality and Data Assurance on Products, Updated December 2021

	% of formulation	Data Quality Score (0-3, 3=good)	Data Assurance Score (Data Quality Score weighted by max % known)	Eye Damage/ Irritation	Skin Corrosion/ Irritation	Skin Sensitization	Repeat Dose Oral Toxicity (LOAEL)	Carcinogenicity	Mutagenicity	ReproDev Toxicity (NOAEL)	Acute Oral Toxicity (LD50)	Acute dermal toxicity (LD50)	Acute Inhal. Toxicity	Aquatic Acute	Aquatic Chronic	LC50 fish	EC50 Daphnia 48 hr	ErC50 Algae	NOEC Chronic Fish	Mobility in Soil	Biodegradation	Bioaccumulative Potential
Bio-Ex ECOPOL A 3%	10-32.5	2.31	0.75																			
Fomtec ENVIRO USP	12-23	2.31	0.53																			
National Foam Avio^{F3} Green KHC 3%	25-61	2.40	1.47																			
National Foam 20-391	5-24	2.33	0.56																			
NRL 502W	33.7	2.37	0.80																			
Solberg Re-Healing Foam RF3 3%	<74.5	2.08	1.55																			

Notes:
Gray bars are visual breaks between products; colored squares indicate data for products (from SDS, experimental, and predicted data).
For square split diagonally: lower/left triangle = data sourced from literature review; upper/right triangle = data sourced from SDS.
Blue = no data found.
White = no search performed.
In general, colors indicate GHS classification (see Appendix B for full details) where green = no toxicity, yellow = low toxicity, orange = moderate toxicity, red = high toxicity, violet = very high toxicity.
Biodegradation: green = readily biodegradable; yellow = not readily biodegradable.
Mobility in soil (based on log Koc and water solubility): green = low, yellow = moderate, red = high.
Bioaccumulation potential (based on log Kow and BCF value): green = low, yellow = moderate, red = high.
Repeat dose toxicity (based on Howe 2007): green = low, yellow = moderate, red = high.
ReproDev Tox: green = >1,000 mg/kg, yellow = >500 mg/kg, orange = >100 mg/kg, red = <100 mg/kg.
NOEC Chronic Fish (based on Howe 2007 using EcoTox LOAEL): green = low, yellow = moderate, red = high.

Table 14. Toxicity Information and Data Quality and Data Assurance on Products Including Incorporation of Highest Hazard GHS Category per Endpoint per Products as Identified in the Constituent-based Approach (Circles), Updated April 2022

	% of formulation	Data Quality Score (0-3, 3=good)	Data Assurance Score (Data Quality Score weighted by max % known)	Eye Damage/ Irritation	Skin Corrosion/ Irritation	Skin Sensitization	Repeat Dose Oral Toxicity (LOAEL)	Carcinogenicity	Mutagenicity	ReproDev Toxicity (NOAEL)	Acute Oral Toxicity (LD50)	Acute dermal toxicity (LD50)	Acute Inhal. Toxicity	Aquatic Acute	Aquatic Chronic	LC50 fish	EC50 Daphnia 48 hr	ErC50 Algae	NOEC Chronic Fish	Mobility in Soil	Biodegradation	Bioaccumulative Potential
Bio-Ex ECOPOL A 3%	10-32.5	2.45	0.80																			
Fomtec ENVIRO USP	12-23	2.55	0.59																			
National Foam 20-391	5-24	2.53	0.61																			
National Foam Avio^{F3} Green KHC 3%	25-61	2.66	1.62																			
NRL 502W	33.7	2.58	0.87																			
Solberg Re-Healing Foam RF3 3%	<74.5	2.46	1.84																			

Notes:
Colored squares indicate data for products (from SDS, experimental, and predicted data).
For square split diagonally: lower/left triangle = data sourced from literature review; upper/right triangle = data sourced from SDS. Circles indicate data from constituents; selected by most hazardous constituent.
Blue = no data found.
White = no search performed.
In general, colors indicate GHS classification (see Appendix B for full details) where green = no toxicity, yellow = low toxicity, orange = moderate toxicity, red = high toxicity, violet = very high toxicity.
Biodegradation: green = readily biodegradable; yellow = not readily biodegradable.
Mobility in soil (based on log Koc and water solubility): green = low, yellow = moderate, red = high.
Bioaccumulation potential (based on log Kow and BCF value): green = low, yellow = moderate, red = high.
Repeat dose toxicity (based on Howe 2007): green = low, yellow = moderate, red = high.
ReproDev Tox: green = >1,000 mg/kg, yellow = >500 mg/kg, orange = >100 mg/kg, red = <100 mg/kg.
NOEC Chronic Fish (based on Howe 2007 using EcoTox LOAEL): green = low, yellow = moderate, red = high.

Table 15. Numerical Representation of the Data in Table 14 as Used to Calculate ToxPi Scores

		ECOPOL A	Buckeye	502W	20-391	AvioGreen	ENVIRO	ReHealing
OccHealth	Ocular Irritation	1	3	1	1	1	1	2
	Dermal Irritation	4	3.5	4	4	3	3	4
	Dermal Sensitization	3	4	4	4	4	4	2
	Carcinogen	4	4	4	4	4	4	4
	Mutagen	4	4	4	4	4	4	4
Mammalian	Oral LOAEL	4	4	3	4	4	4	4
	DART LOAEL	5	4	5	4	2	3	3
	Oral LD50	5	5	5	5	5	5	5
	Dermal LD50	3	5	5	5	5	5	5
	Inhalation Tox	5	1	5	1	5	1	2
AquaticEco	Aquatic Acute	4	5	4	4	3	4	4
	Aquatic Chronic	4	5	4	4	5	4	2
	Fish LC50	5	5	5	5	4	5	5
	Daphnia EC50	5	5	5	5	4	5	5
	Algae EC50	5	5	5	5	3	5	5
	Fish NOEC	5	4	4	2	4	2	2
PBF&T	Soil Mobility	2	2	2	2	2	2	2
	Biodegradation	5	2	5	5	5	5	5
	Bioaccumulation	5	5	2	5	5	5	5
	ToxPi Score	0.66	0.55	0.52	0.48	0.4	0.33	0.3

Notes:
See main text for description of each endpoint.
Categorization scores of 1 represent high hazard, 5 represents low hazard in the APHC (Howe et al., 2007) and GHS inhalation toxicity endpoints, 4 represents low hazard in the other GHS categories.
ToxPi scales the overall scores per category and provides a relative hazard score.
Mismatches in data between SDS and test/literature/predicted data are represented by averages.

7.6 Relative Toxicity Ranking of Products

ToxPi ranking indicates that Solberg Re-Healing Foam RF3 3% is the most hazardous product relative to the other products (Table 16, Figure 1 to Figure 4). BIOEX ECOPOL A scores the least hazardous with a moderate data assurance score (Table 16, Figure 1). The short-chain PFAS-containing reference product Buckeye Platinum Plus C6 scores the second least hazardous but also has the lowest data assurance score (Table 16, Figure 1). Importantly, Solberg Re-Healing Foam RF3 3% also has the highest data assurance score indicating that this relative ranking comes from the best quality data weighted by maximum proportion of chemicals disclosed in the SDS (Table 14 and Table 15).

Table 16. ToxPi Scores for Products Based on Quantified GHS Categories, Relative Rank, and Data Assurance Scores

Product	ToxPi Score	Rank	Data Assurance Score
ECOPOL A	0.66	7 (least hazardous)	0.80
Buckeye	0.55	6	0.49
NRL 502W	0.52	5	0.87
20-391	0.48	4	0.61
AvioGreen	0.40	3	1.62
Enviro USP	0.33	2	0.59
Re-Healing	0.30	1 (most hazardous)	1.84

The ToxPi scores are depicted using radar plots that visualize how individual groups of hazard/toxicity endpoints influence scoring (Figure 1 to Figure 4). Nearly all of the PFAS-free AFFFs show high relative hazards (smaller slices) associated with occupational health and aquatic ecotoxicity, low relative hazards (larger slices) for PBF&T, and varying mammalian relative hazards (Figure 1 to Figure 4).

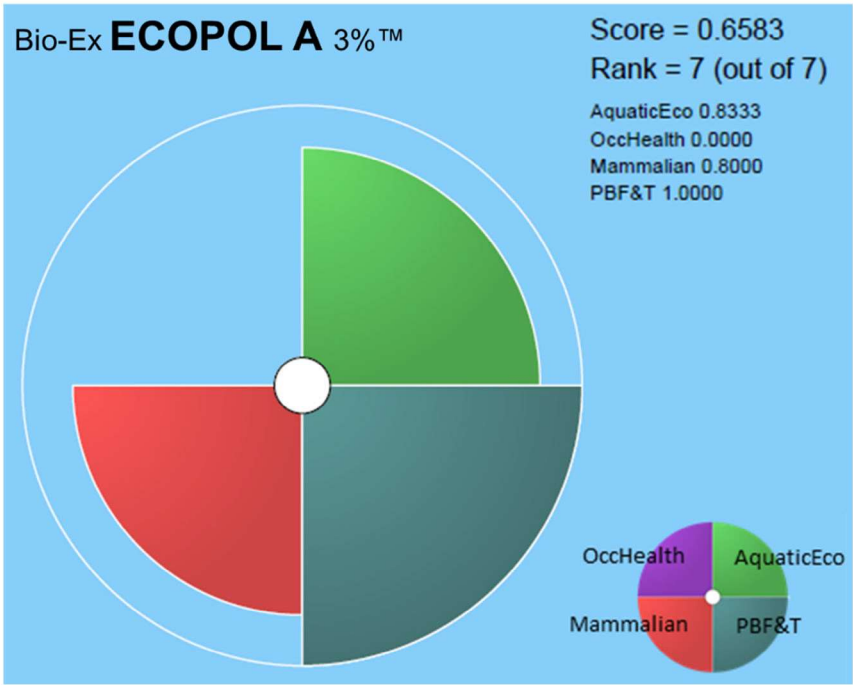


Figure 1. ToxPi Radar Plot for ECOPOL A Using Quantified GHS Categories from Table 14 and Table 15

Note: Categorical score breakdown is provided below the total score and rank. Color key indicates slice category. Slice sizes and relative hazard are inversely related (e.g., smaller slice = higher hazard).

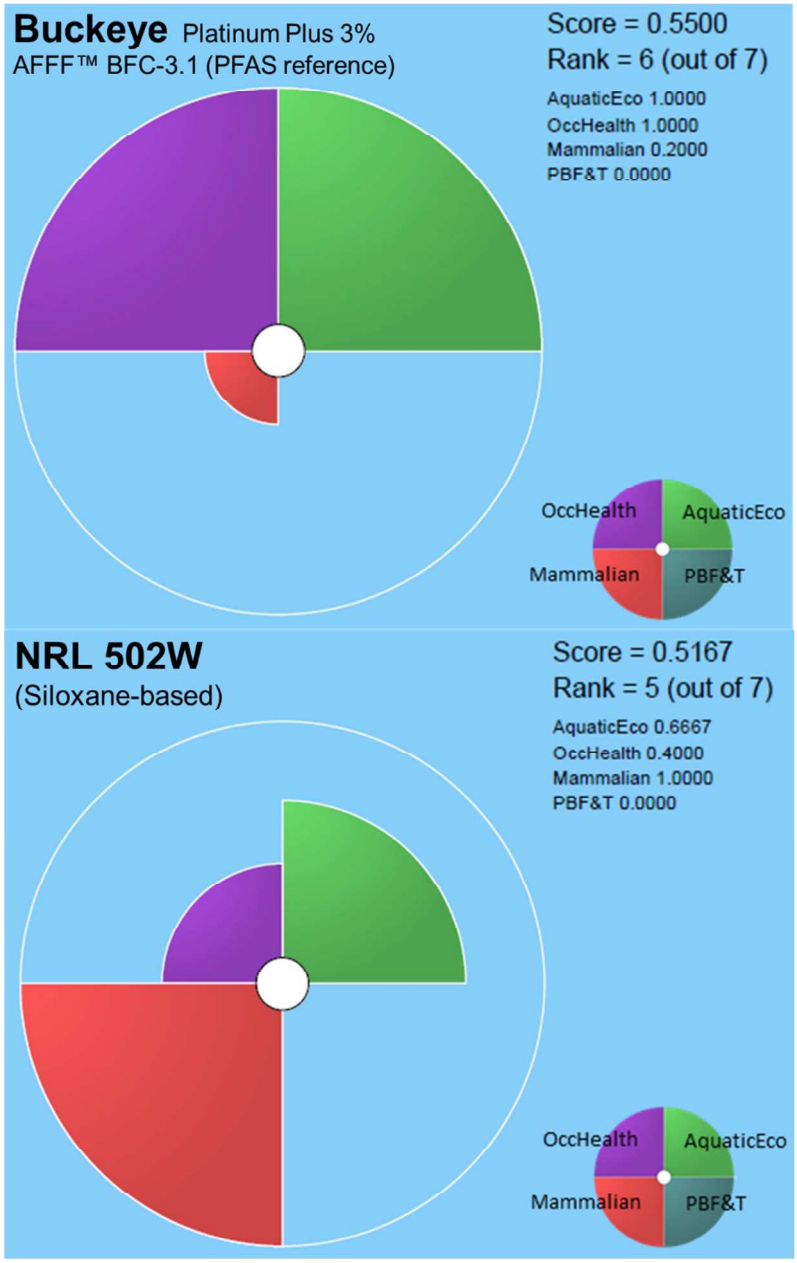


Figure 2. ToxPi Radar Plots for Buckeye and NRL 502W Using Quantified GHS Categories from Table 14 and Table 15

Note: Categorical score breakdown is provided below the total score and rank. Color key indicates slice category. Slice sizes and relative hazard are inversely related (e.g., smaller slice = higher hazard).

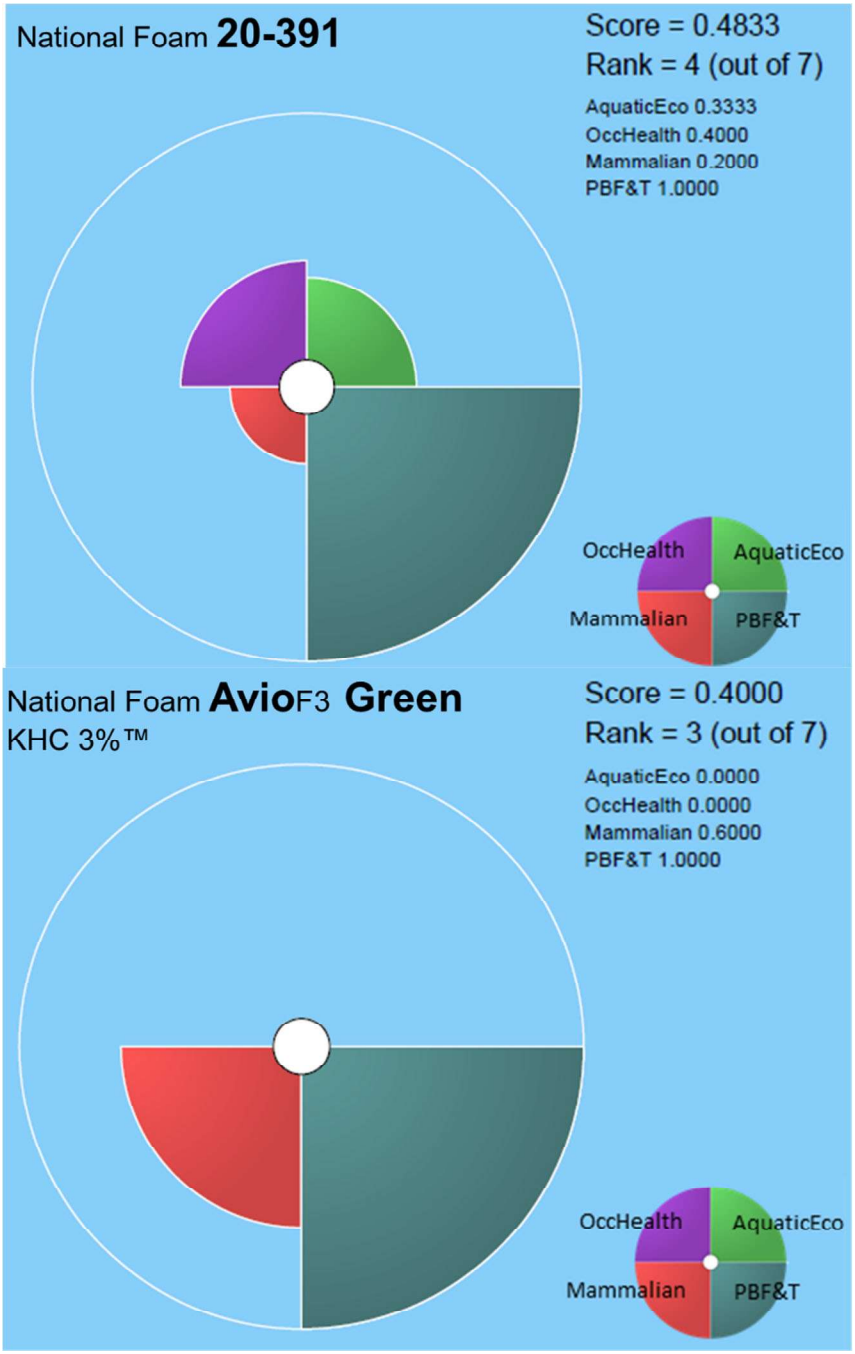


Figure 3. ToxPi Radar Plots for 20-391 and AvioGreen Using Quantified GHS Categories from Table 14 and Table 15

Note: Categorical score breakdown is provided below the total score and rank. Color key indicates slice category. Slice sizes and relative hazard are inversely related (e.g., smaller slice = higher hazard).

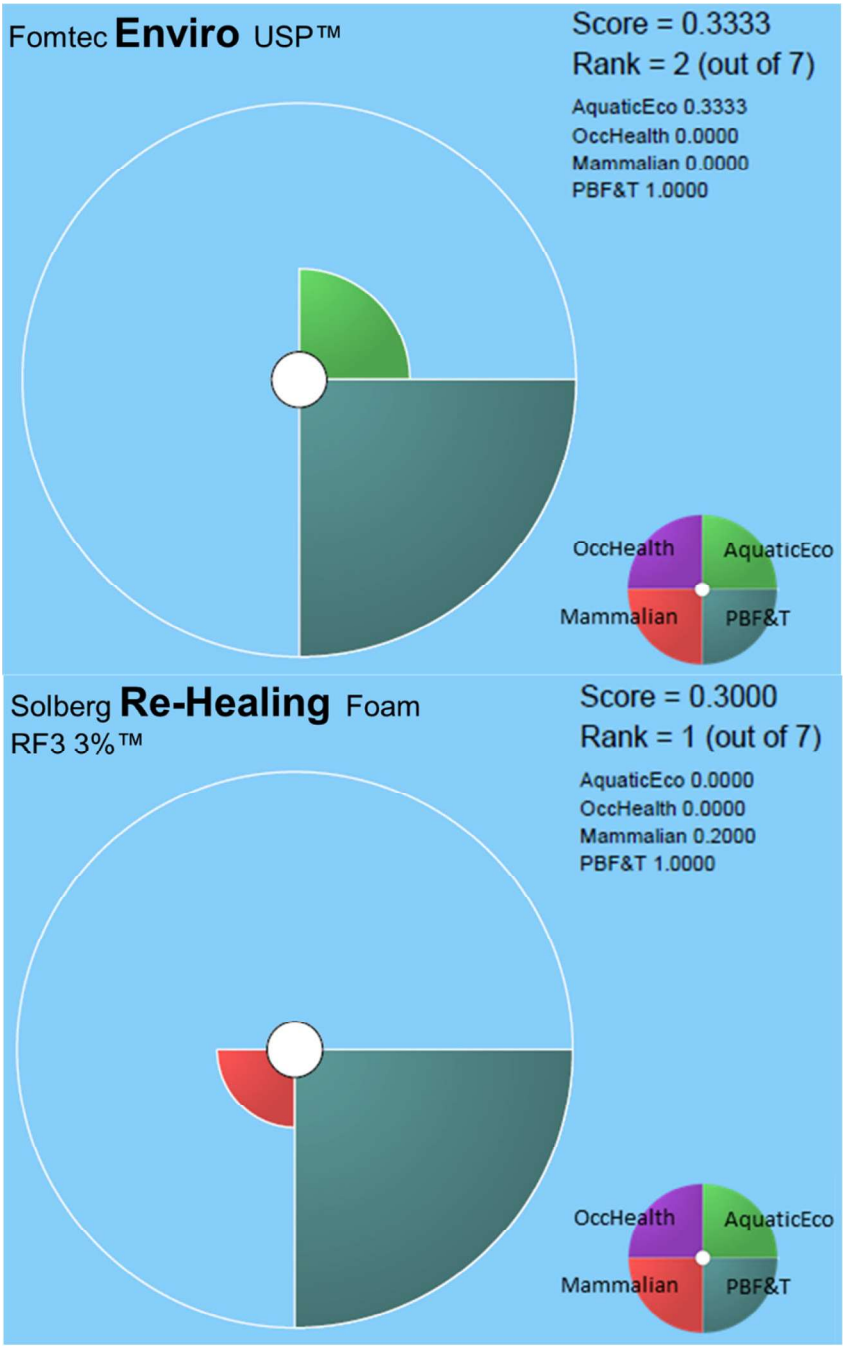


Figure 4. ToxPi Radar Plots for Enviro and Re-Healing Using Quantified GHS Categories from Table 14 and Table 15

Note: Categorical score breakdown is provided below the total score and rank. Color key indicates slice category. Slice sizes and relative hazard are inversely related (e.g., smaller slice = higher hazard).

Similarity and difference between mammalian, occupational health, aquatic ecotoxicity, and PBF&T hazards across the products are depicted in Figure 5 with a complete-linkage, hierarchical cluster dendrogram. The first and second nodes split Buckeye and NRL 502W away from the remaining five PFAS-free AFFFs. The radar plots indicate this is due to the potential PBF&T hazards associated with NRL 502W and the low occupational health and aquatic ecotoxicity hazards of Buckeye. Within the remaining cluster of PFAS-free AFFFs, the similarity appears to be large slices (low hazard) for PBF&T, and then varying medium-to-small slices (medium-to-high hazard) for all other categories.

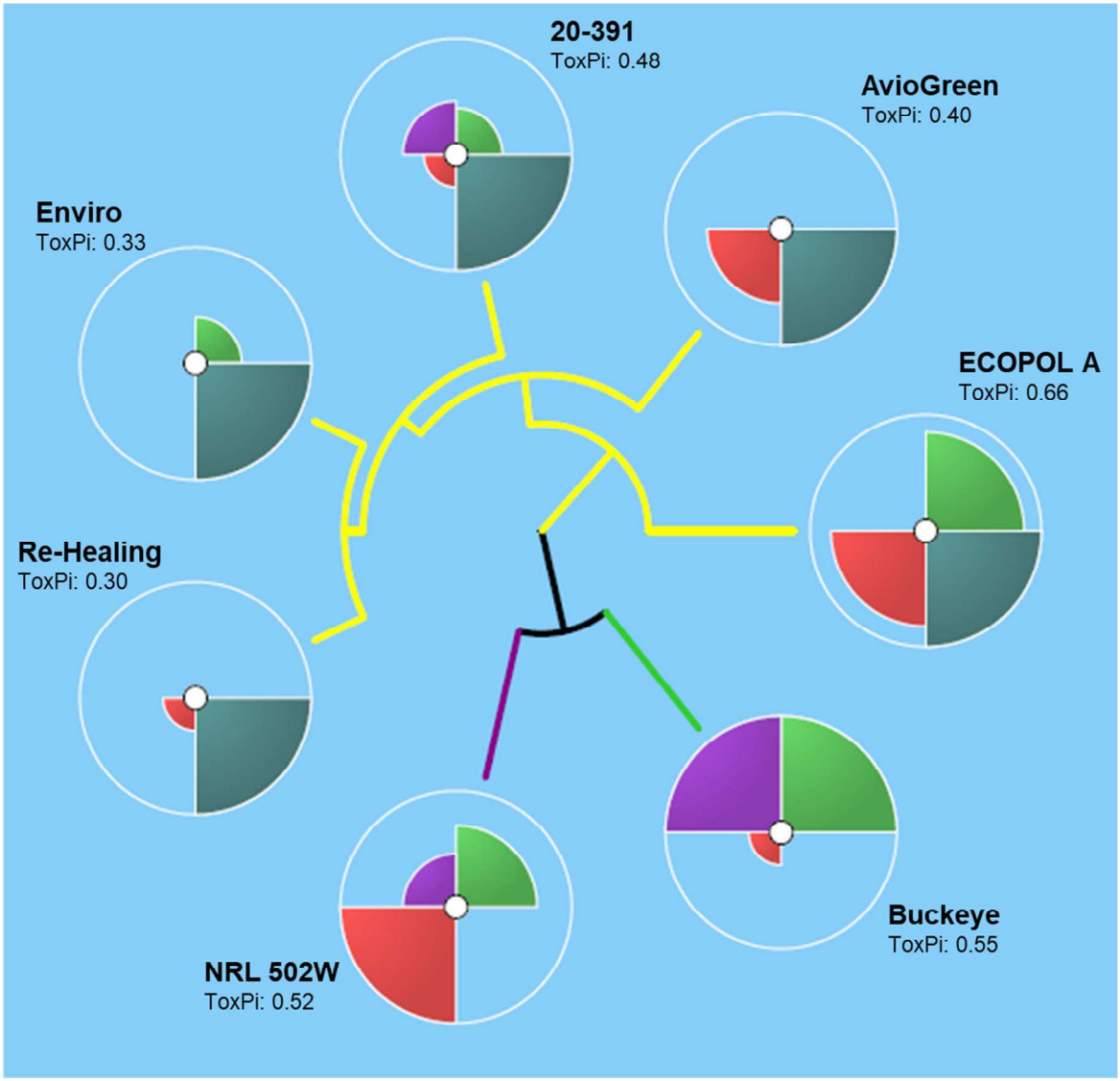


Figure 5. Circular Dendrogram of Three Agglomerative Hierarchical Clusters (Complete Linkage Method; Indicated by Branch Color) of ToxPi Slice Data for PFAS-free and PFAS-Containing AFFFs

Note: Slice sizes and relative hazard are inversely related (e.g., smaller slice = higher hazard).

8 DISCUSSION

8.1 Product Summaries

A compilation of the data from literature review, manufacturer-supplied SDS, predictive methods, and direct whole-product toxicity testing is presented in paragraph 7.5 and Table 13 and Table 14. Complete constituent profiles are available in Addendum 1. A key consideration for these products is that known components of each AFFF range from 5% to 74.5%. As such, up to 95% of a product has not been disclosed by the manufacturer. Tables of summarized data include data assurance scores, which should be interpreted as how much assurance can be placed on the completeness and accuracy of the data for that product. Downstream decisions and inference should be made with confidence that stems from assurance in the accuracy of the supporting data, which is why it is important to include and highlight the data assurance score. See paragraph 8.5.1 for a discussion of major areas of concern with these products.

8.1.1 BIOEX ECOPOL A 3% FFF

There are five constituents listed in the SDS for BIOEX ECOPOL A 3%, but only one (DGBE) was described using a CASRN, meaning that the four other constituents have not been fully identified or characterized in this Toxicity Assessment. BIOEX has disclosed between 10% and 32.5015% of the product, leaving 68.5% to 90% of the product unknown.

In-house aquatic toxicity in bacteria resulted in a classification as GHS category 2 or moderately toxic. In-house skin irritation testing resulted in a classification as a non-irritant. In-house acute and subacute (28-day repeat dose) oral exposure in CD-1 mice result in non-categorized GHS, low concern toxicity classification as well as a negative micronucleus assay response. Product testing in aquatic systems returns category 3 and category 2 GHS classifications for acute and chronic fish, invertebrate, and algae exposures (category. 2).

8.1.2 Fomtec Enviro USP

There are four constituents listed in the SDS for Fomtec Enviro USP and all are described using a CASRN. Fomtec has disclosed between 12 and 23% of the product, leaving 77% to 88% of the product unknown.

In-house aquatic toxicity in bacteria resulted in a classification as GHS category 2 or moderately toxic. In-house skin irritation testing resulted in a classification as a non-irritant. In-house acute and subacute (28-day repeat dose) oral exposure in CD-1 mice result in non-categorized GHS, low concern toxicity classification as well as a negative micronucleus assay response. Product testing in aquatic systems returns category 3 GHS classifications for acute and chronic fish, invertebrate, and algae exposures.

8.1.3 National Foam 20-391

There are three constituents listed in the SDS for National Foam 20-391. Two are described using a CASRN, but one does not have an assigned CASRN. Instead, that component is

described with a European Chemical Number (ECN). National Foam has disclosed between 5% and 24% of the product, leaving 76% to 95% of the product unknown.

In-house aquatic toxicity in bacteria resulted in a classification as GHS category 3 or slightly toxic. In-house skin irritation testing resulted in a classification as a non-irritant. In-house acute and subacute (28-day repeat dose) oral exposure in CD-1 mice result in non-categorized GHS, low concern toxicity classification as well as a negative micronucleus assay response. Product testing in aquatic systems returns category 3 and category 2 GHS classifications for acute and chronic fish, invertebrate, and algae exposures (category 2).

8.1.4 National Foam Avio^{F3} Green KHC 3%

There are four constituents listed in the SDS for National Foam Avio^{F3} Green KHC 3% and all are described using a CASRN. National Foam has disclosed between 25% and 61% of the product, leaving 39% to 75% of the product unknown.

In-house aquatic toxicity in bacteria resulted in a classification as GHS category 1 or highly toxic. In-house skin irritation testing resulted in a classification as a non-irritant. In-house acute and subacute (28-day repeat dose) oral exposure in CD-1 mice result in non-categorized GHS, low concern toxicity classification as well as a negative micronucleus assay response. Product testing in aquatic systems returns non-categorized, category 3 and category 2 GHS classifications for acute and chronic fish (category 2 or 3), invertebrate (non-categorized or category 3), and algae exposures (category 2).

8.1.5 NRL 502W

There are three constituents listed in the SDS for NRL 502W and all are described using a CASRN. NRL has disclosed 33.7% of the product on the SDS, but disclosed that the remainder was water on the bottle and via direct communication with APHC.

In-house aquatic toxicity in bacteria suggests NRL 502W is practically nontoxic (i.e., below the level for cytotoxicity). In-house skin irritation testing resulted in a classification as a non-irritant. In-house acute and subacute (28-day repeat dose) oral exposure in CD-1 mice result in non-categorized GHS acute, moderate repeat dose concern toxicity classification as well as a negative micronucleus assay response. Product testing in aquatic systems returns non-categorized and category 3 GHS classifications for acute and chronic fish and invertebrate exposures.

8.1.6 Solberg Re-Healing Foam RF3 3%

There are eight constituents listed in the SDS for Solberg Re-Healing Foam RF3 3% and all are described using a CASRN. Solberg has disclosed up to 74.5% of the product, leaving 25.5% of the product unknown. Based on disclosed, protected information, Solberg Re-Healing Foam RF3 3% should be removed from consideration due to an inability to meet the current (draft) MILSPEC for PFAS-Free AFFF replacements (see Addendum 2).

In-house aquatic toxicity in bacteria resulted in a classification as GHS category 3 or slightly toxic. In-house skin irritation testing resulted in a classification as a non-irritant. In-house acute and subacute (28-day repeat dose) oral exposure in CD-1 mice result in non-categorized GHS, low concern toxicity classification as well as a negative micronucleus assay response. Product testing n aquatic systems returns non-categorized and category 3 GHS classifications for acute fish, invertebrate, and algae exposures.

8.2 Relative Ranking

As PFAS-containing AFFF are phased out, there will be some hazard/toxicity tradeoffs. Specifically, it is likely that there will be increased short-term aquatic ecotoxicity and irritation hazards, but reduced mammalian toxicity and PBF&T hazards (paragraph 7.6). To illustrate this tradeoff, ECOPOL A—the product with the highest ToxPi score (lowest overall relative hazard)—has higher occupational health hazards (associated with ocular and dermal irritation), but low relative hazards for mammalian toxicity, aquatic ecotoxicity, and PBF&T. Buckeye—the PFAS-containing AFFF comparator—has high mammalian toxicity and PBF&T hazard, which is a starkly different pattern than the overall profile of the PFAS-free AFFFs.

There are hazard tradeoffs within the assessed PFAS-free products, as well. Importantly, NRL 502W shows the most toxicity via *in vivo* testing but is one of the least hazardous PFAS-free AFFF replacement in the ToxPi scoring due to its low aquatic ecotoxicity scores. In addition to the hazard assessment summarized in paragraph 8.1, NRL 502W contains siloxane-type constituents, which present the potential for environmental persistence and bioaccumulation. NRL 502W is unlikely to be more persistent or bioaccumulative than legacy PFAS (e.g., perfluorooctane sulfonate (PFOS) or perfluorooctanoate (PFOA)), but may impact the ability to classify all PFAS-free AFFFs as having low or no PBF&T hazard.

ToxPi ranking itself should not be used as a standalone guide for decision makers. It can be difficult to interpret the ToxPi ranking—based on averages of a wide range of endpoints—as a single answer to relative ranking. Tradeoffs of hazard and toxicity based on variable exposure scenarios requires risk consideration for final ranking or decisions.

8.3 Uncertainty Analysis

Inclusion of data assurance scores adds another layer to interpreting the nuance of relative ranking of these PFAS-free AFFFs. As an example, Buckeye has a lower data assurance score than NRL 502W, but they have similar ToxPi scores. When there is ambiguity about the ranking or clusters based on ToxPi scoring, an increased data assurance score should be used to either break a tie or create a tie and justify further exploration or testing. In the case of NRL 502W and Buckeye, their ranks (5^{th} and 6^{th}, respectively) and data assurance scores represent a tie and when compared to ECOPOL A, with a lower hazard rank (7^{th}), but lower data assurance score, should be considered on an equivalent basis.

Disclosed, protected information (Addendum 2) is based on targeted and non-targeted methods and focused on positively identifying chemicals, so has little overall impact on uncertainty analysis from a quantitative or concentration-based standpoint.

8.4 Regulations and Standards

Existing regulations and standards are listed in Table 17. Regulations or standards were found for five of the 21 disclosed chemicals. Most of the regulations are tied to the inhalable fraction (as dust) or as a vapor, but all of the disclosed chemicals have very low vapor pressures and all of the chemicals are present in an aqueous solution. As these AFFFs are used as a 3% foam, there is some, albeit low, risk of inhalation exposure to mists created during application of foam.

Table 17. Individual Constituent Regulations and Standards

Compound	CASRN	OSHA PEL	NIOSH REL	ACGIH TLV	EPA IRIS	Other
DBGE	112-34-5	NA	NA	TWA = 10 ppm (inhalable fraction, vapors)	RfC_pr = 0.0001 mg/m³, RfDo_pr = 0.03 mg/kg-d, RfC_pr_schr = 0.001 mg/m³, RfDo_pr_schr = 0.3 mg/kg-d	LTEL = 67.5 mg/m^{3,}; STEL = 101.2 mg/m^{3,} DOD MEG_1hourCRIT_air = 2,500 mg/m³, MEG_1hourMARG_air = 600 mg/m³, MEG_1hourNEG_air = 100 mg/m³, MEG_1yearNEG5L_water = 4.2 mg/L, MEG_1yearNEG_air = 0.000685 mg/m³, MEG_1yearNEG_soil = 59600 mg/kg; DOE PAC_1 = 200 mg/m³, PAC_2 = 220 mg/m³, PAC_3 = 1300 mg/m³; EPA SL_Indus-air = 0.44 µg/m³, SL_Res-air = 0.1 µg/m³, SL_Tap = 600 µg/L, SSL_Indus-soil = 24,000 mg/kg, SSL_GW-risk = 0.13 mg/kg, SSL_Res-soil = 1,900 mg/kg, VISL_Com-IndoorAir = 0.438 µg/m³, VISL_Res-IndoorAir = 0.104 µg/m³; NSF STEL = 8 mg/L, SPAC = 0.06 mg/L, TAC = 0.6 mg/L; States with regulations: Arizona, Michigan, North Carolina, Nevada, Oregon, Texas
Alkylbetaine	not disclosed	NA	NA	NA	NA	NA
Alkylsulfate	not disclosed	NA	NA	NA	NA	NA
Amphoteric Surfactant	not disclosed	NA	NA	NA	NA	NA
Preservative	not disclosed	NA	NA	NA	NA	NA
HG	107-41-5	NA	25 ppm or 125 mg/m^3 (instantan-eous)	STEL = 10 mg/m3 (inhalable particulate, aerosol) STEL = 50 ppm (vapor) TWA = 25 ppm (vapor)	NA	DOD MEG_1hourCRIT_air = 1,500 mg/m³, MEG_1hourMARG_air = 125 mg/m³, MEG_1hourNEG_air = 50 mg/m³; DOE PAC = PAC_1 = 11 mg/m³, PAC_2 = 120 mg/m³, PAC_3 = 730 mg/m³; NSF SPAC = 0.01 mg/L, TAC = 0.01 mg/L; States with regulations: California, Idaho, Michigan, Texas
AS C12-14 TEA	90583-18-9	NA	NA	NA	NA	NA
AES C12-14 2.5EO Na	68891-38-3	NA	NA	NA	NA	NA
AO C12-14	308062-28-4	NA	NA	NA	NA	NA
CAPHS C8-18	ECN 939-455-3	NA	NA	NA	NA	NA
AO C12	1643-20-5	NA	NA	NA	NA	NA
AS C12 Na	151-21-3	NA	NA	NA	NA	DOD MEG_1hourCRIT_air = 500 mg/m³, MEG_1hourMARG_air = 6 mg/m³, MEG_1hourNEG_air = 1 mg/m³; DOE PAC_1 = 3.9 mg/m³, PAC_2 = 43 mg/m³, PAC_3 = 260 mg/m³; NSF SPAC = 0.7 mg/L, TAC = 7 mg/L
AO C14	3332-27-2	NA	NA	NA	NA	NA
DG	68515-73-1	NA	NA	NA	NA	NA
PDMS EO	67674-67-3	NA	NA	NA	NA	NA

Compound	CASRN	OSHA PEL	NIOSH REL	ACGIH TLV	EPA IRIS	Other
CAPB C12	61789-40-0	NA	NA	NA	NA	NA
AS C12 TEA	139-96-8	NA	NA	NA	NA	NA
AES C9-111-3EO Na	96130-61-9	NA	NA	NA	NA	NA
CAPHS C12	68139-30-0	NA	NA	NA	NA	NA
Starch	9005-25-8	15 mg/m^3 (total dust), 5 mg/m^3 (respirable fraction)	10 mg/m^3 (total), 5 mg/m^3 (resp)	TWA = 10 mg/m^3	NA	DOD MEG_1hourCRIT_air = 500 mg/m^3, MEG_1hourMARG_air = 500 mg/m^3, MEG_1hourNEG_air = 30 mg/m^3, MEG_1yearNEG_air = 2.45 mg/m^3, MEG_14dayNEG_air = 2.45 mg/m^3, MEG_8hourNEG_air = 10 mg/m^3; States with regulations: California, Oregon
Sucrose	57-50-1	15 mg/m^3 (total dust), 5 mg/m^3	10 mg/m^3 (total), 5 mg/m^3 (resp)	TWA = 10 mg/m^3	NA	DOD MEG_1hourCRIT_air = 500 mg/m^3, MEG_1hourMARG_air = 50 mg/m^3, MEG_1hourNEG_air = 30 mg/m^3, MEG_1yearNEG_air = 2.45 mg/m^3, MEG_14dayNEG_air = 2.45 mg/m^3, MEG_8hourNEG_air = 10 mg/m^3; States with regulations: California, Oregon

Legend:
PEL = permissible exposure limit
REL = recommended exposure limit
IRIS = Integrated Risk Information System
NA = not applicable
mg/m^3 = milligrams per cubic meter
mg/kg = milligrams per kilogram
TWA = time weighted average
STEL = short term exposure limit
ppm = parts per million
MEG = military exposure guidelines
LTEL = long term exposure limit

8.5 Summary and Conclusions

Based on a thorough literature review, estimation techniques, and incorporation of testing data with whole products, PFAS-free AFFFs will present a different toxicity profile than current PFAS-containing AFFFs. Notable trade-offs detected in this assessment are reduced mammalian toxicity, persistence, and likelihood of bioaccumulation, but increased aquatic ecotoxicity and irritation hazards. There does remain uncertainty associated with the amount of information available in SDS provided by manufacturers. See Addendum 2 for CBI-derived summary and conclusions.

8.5.1 Major Areas of Concern

The major areas of concern for potential human health toxicity of all tested products are acute ocular and dermal irritation of concentrated (undiluted) products. Dermal irritation tests performed at 3% w/v concentration found the AFFFs to be non-irritating. Additional testing or PPE considerations are recommended for workers handling the products as concentrates. *In vitro* and *in vivo* testing data using 3% w/v is directly relevant to potential occupational hazards associated with exposure to the 3% foam formulation. Standard PPE and engineering controls will likely reduce human exposures and prevent hazards to skin or eyes.

Acute and subacute (28-day repeat dose) oral toxicity testing in mice for the six products were completed at APHC. Follow up work assessing repeat dose toxicity and reproductive and developmental toxicity are scheduled for 2022 – 2024. Acute oral toxicity in mice is uncategorized according to GHS standards. Only NRL 502W had moderate concern using the LOAEL metrics in the Howe et al. (2007) framework at approximately 1.25% dilution. All others were low concern for LOAEL type endpoints after subacute, 28-day repeat exposures.

The major environmental and ecological toxicity concerns for the six products are impacts on aquatic systems at high concentrations. Acute aquatic toxicity in bacteria is moderate to high for all products except NRL 502W (siloxane-based). Additional aquatic toxicity testing in other species such as algae, daphnia, and fish is recommended for diluted concentrations expected to be released to the environment. Data from standardized aquatic testing systems leads to low to moderate concern or GHS categories of uncategorized, category IV, III, and II in algae, daphnids, and fish (SERDP 2021 Winter Symposium (Hoverman 2021, Kuperman 2021, Suski 2021, Wirth 2021, Wu 2021)) for all products. Moderate concern and GHS category III (yellow in stoplight visuals) was the predominant interpretation for 4 of the 6 products. Solberg Re-Healing Foam RF3 3% and NRL 502W were either predominantly uncategorized/category IV/low concern or a 50:50 split between green/yellow based on SDS vs. experimental data mismatches. Based on disclosed, protected information, Solberg Re-Healing Foam RF3 3% should be removed from consideration due to an inability to meet the current (draft) MILSPEC for PFAS-Free AFFF replacements and release volumes should be considered to better understand hazards of unreported but positively identified chemicals (APHC 2021, Addendum 2). The largest enduring area of concern about bioaccumulation or persistence of these products are the unknown fractions of each mixture and the siloxane constituent(s) of NRL 502W (Table 18).

Table 18. Environmental Persistence (P), Bioaccumulation (B), and Toxicity (T) Table

Product	PBT	Comments
BIOEX ECOPOL A 3%	T	Moderate aquatic toxicity. Low bioaccumulation, high mobility, high rate of biodegradation.
Fomtec Enviro USP	T	Moderate aquatic toxicity. Low bioaccumulation, moderate mobility, high rate of biodegradation.
National Foam Avio^{F3} Green KHC 3%	T	Moderate aquatic toxicity. Low bioaccumulation, high mobility, high rate of biodegradation.
National Foam 20-391	T	Moderate aquatic toxicity. Low bioaccumulation, high mobility, high rate of biodegradation.
NRL 502W (Siloxane-based)	B	Low aquatic toxicity. High mobility. One constituent with predicted category 1 bioaccumulation and uncertain product bioaccumulation. High rate of biodegradation.
Solberg Re-Healing Foam RF3 3%	T	Moderate aquatic toxicity. Low bioaccumulation, high mobility, high rate of biodegradation.

Note: Primary areas of concern related to PBT are captured in this table. Data originate from Table 14 (paragraph 7.5) and focus on aquatic toxicity (not human health) of the products.

9 RECOMMENDATIONS

Overall, relative ranking and cluster analysis has identified patterns in product toxicity that can inform potential downselection decisions. Based on fairly equivalent rankings of PBF&T, and occupational, mammalian, and aquatic toxicity, five of the alternative products (ECOPOL A, NRL 502W, NFD 20-391, AvioGreen, and Enviro) are fairly equivalent, with relative tradeoffs and ranks based on each of those categories. Using current data, ECOPOL A is the PFAS-free AFFF with the lowest overall hazard, but presents a similar hazard profile with other PFAS-free AFFF replacement products. There is concern about the identification, concentration, and potential environmental persistence of siloxane in NRL 502W and potential impacts of pending legislation on siloxanes (EPA 2018b; CFR 702-41), which contributes to its unique hazard profile among replacement products. Solberg Re-Healing Foam RF3 3% ranks as the most hazardous with a high data assurance score. Furthermore, based on disclosed, protected information, Solberg Re-Healing Foam RF3 3% should be removed from consideration due to an inability to meet the current (draft) MILSPEC for PFAS-free AFFFs (APHC 2021, Addendum 2). Use of proper PPE, engineering controls, and adherence to local, state, and federal guidelines for occupational exposure and hazardous waste disposal are necessary.

Related to human health, many of the reported constituents and similarly structured compounds are used at low concentrations in household products, soaps, cosmetics, and other products that are generally thought safe. Testing of products for ocular and dermal irritation/sensitization

is recommended due to likelihood of occupational exposure. Acute and subacute toxicity appear to be of low concern for direct ingestion based on exposures in mice.

Environmental health generalizations rely on assuming that the unknown portions of the products do not contain metals, halogenated organics, or other molecules known to be persistent or bioaccumulative. Based on disclosed, protected information, release volumes should be considered to better understand hazards of unreported but positively identified chemicals (APHC 2021, Addendum 2). Given the properties of the chemicals described in this Toxicity Assessment and their proposed intentional use, they are designed to perform well as water soluble surfactants and smothering agents (as a foam formulation). In concentrated form, these AFFF products are likely to be acutely toxic to environmental (aquatic) receptors. When used at the intended dilute concentration (generally 3%), these formulations are less likely to be acutely toxic and, while mobile in soil and water, are generally not expected to be persistent in the environment. ECOPOL A appears to be least hazardous PFAS-free AFFF overall, but does not necessarily have improved environmental toxicity or hazard over the PFAS-containing reference, most likely due to tradeoffs in toxicity (ECOPOL A is more toxic) against persistence (the PFAS in Buckeye persist in biodegradation tests).

The following additional testing is recommended:

1. Skin irritation/sensitization with products.
2. Reproductive and developmental toxicity testing in mammals.
3. Chronic ecotoxicity testing and/or environmental persistence data relevant to site-specific risk assessment data needs.
4. Assessment of potential combustion breakdown products and associated toxicity testing following TG 389 (APHC 2021).

Importantly, many of the tests mentioned above are underway at APHC or via SERDP-ESTCP collaborators, and this phased Toxicity Assessment represents the status of information available as of April 2022.

Generalizations on the human toxicity, environmental fate, persistence, bioaccumulation, and ecotoxicity of fluorine-free AFFFs can be made from the information provided by the manufacturers, available literature, prediction methods, and direct product toxicity testing and have been summarized in this document. Full disclosure SDS from each manufacturer would increase assurance in the values used to calculate rankings, and therefore increase confidence in decisions informed by ranking.

10 POINTS OF CONTACT

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APPENDIX A

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APPENDIX B

The Globally Harmonized System (GHS) Hazard Categories

GHS is the acronym for the Globally Harmonized System of Classification and Labeling of Chemicals. The GHS attempts to establish international consensus for defining health, physical, and environmental hazards of chemicals; creating a classification process for comparison with defined hazard criteria; and communicating hazard information and protective measures on labels and Safety Data Sheets (SDS, formerly known as Material Safety Data Sheets). The GHS attempts to reduce differences among levels of protection for workers established by the different countries and reduce regulatory burden and barriers to commerce while establishing consistent standards for classification. The GHS is the result of an international mandate adopted in the 1992 United Conference on Environment and Development, often called the "Earth Summit." The harmonization and classification of chemicals was one of six program areas endorsed by the United Nations General Assembly to strengthen international efforts in the environmentally sound management of chemicals.

While there are several aspects of the GHS, the one most important area for our purposes is classification of chemicals into various hazard categories based upon their effects and the route of exposure. Tabular extracts of the criteria for acute toxicity (both oral and inhalation), dermal, and ocular effects are included below. More information can be found in the original source (OSHA 2012).

Table B-1. GHS Acute Toxicity

	Category 1	Category 2	Category 3	Category 4	Category 5
Oral (mg/kg)	≤5	>5 ≤50	>50 ≤300	>300 ≤2,000	Criteria: -Anticipated LD_{50} between 2,000 and 5,000 mg/kg. -Indication of significant effects in humans. -Any mortality in Category 4. -Significant clinical signs in Category 4. -Indications from other studies. *If assignment to a more hazardous class is not warranted.
Dermal (mg/kg)	≤50	>50 ≤200	>200 ≤1,000	>1,000 ≤2,000	
Gases (ppm)	≤100	>100 ≤500	>500 ≤2,500	>2,500 ≤5,000	
Vapors (mg/L)	≤0.5	>0.5 ≤2.0	>2.0 ≤10	>10 ≤20	
Dusts & Mists (mg/L or g/m^3)	≤0.05	>0.05 ≤0.5	>0.5 ≤1.0	>1.0 ≤5	

Legend:
mg/kg = milligrams per kilogram
LD_{50} = dose expected to result in 50% lethality
Note: Colors are used in summary tables throughout this document to quickly identify GHS hazard category.

Table B-2. GHS Skin Corrosion/Irritation

Skin Corrosion Category 1			Skin Irritation Category 2	Mild Skin Irritation Category 3	Not categorized
Destruction of dermal tissue; visible necrosis in at least one animal.			Reversible adverse effects in dermal tissue Draize score: ≥ 2.3, <4.0, or persistent inflammation	Reversible adverse effects in dermal tissue Draize score: ≥ 1.5, <2.3	Corrosion and irritation not observed
Subcategory 1A	**Subcategory 1B**	**Subcategory 1C**			
Exposure < 3 minutes Observation < 1 hour	Exposure < 1 hour Observation < 14 days	Exposure < 4 hours Observation < 14 days			

Note: Colors are used in summary tables throughout this document to quickly identify GHS hazard category.

Table B-3. GHS Eye Effects

<table>
<tr><th>Category 1
Serious Eye Damage</th><th colspan="2">Category 2
Eye Irritation</th><th>Not categorized</th></tr>
<tr><td rowspan="3">Irreversible damage 21 days after exposure
Draize score:
Corneal opacity ≥ 3
Iritis ≥ 1.5</td><td colspan="2">Reversible adverse effects on cornea, iris, conjunctiva
Draize score:
Corneal opacity ≥ 1
Iritis > 1
Redness ≥ 2
Chemosis ≥ 2</td><td rowspan="3">Non-irritating</td></tr>
<tr><td>Subcategory 2A</td><td>Subcategory 2B</td></tr>
<tr><td>Irritant
Reversible in 21 days</td><td>Mild irritant
Reversible in 7 days</td></tr>
</table>

Note: Colors are used in summary tables throughout this document to quickly identify GHS hazard category.

Table B-4. GHS Acute and Chronic Aquatic Toxicity

Acute Category I	Acute Category II	Acute Category III	Not categorized
Acute toxicity ≤ 1.00 mg/L	Acute toxicity 1.00 – 10.0 mg/L	Acute toxicity 10.0 – 100 mg/L	Acute toxicity > 100 mg/L
Chronic Category I	**Chronic Category II**	**Chronic Category III**	**Chronic Category IV**
Biodegradation half-life >7 d Acute toxicity ≤ 1.00 mg/L log Kow ≥ 4 unless BCF < 500	Biodegradation half-life >7 d Acute toxicity 1.00 – 10.0 mg/L log Kow ≥ 4 unless BCF < 500 unless chronic toxicity > 1 mg/L	Biodegradation half-life >7 d Acute toxicity 10.0-100.0 mg/L log Kow ≥ 4 unless BCF < 500 unless chronic toxicity > 1 mg/L	Biodegradation half-life >7 d Acute toxicity > 100.0 mg/L log Kow ≥ 4 unless BCF < 500 unless chronic toxicity > 1 mg/L

Legend:
d = days
Note: Must meet all criteria per category; Colors are used in summary tables throughout this document to quickly identify GHS hazard category.

GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
ACURO	Animal Care and Use Review Office
AFFF	Aqueous Film Forming Foams
APHC	U.S. Army Public Health Center
AR	Army Regulation
ASTM	American Society for Testing and Materials
ATSDR	U.S. Department of Health and Human Services' Agency for Toxic Substances and Disease Registry
bp	Boiling Point
°C	Degrees Celsius
CASRN	Chemical Abstracts Service Registry Number
CBI	Confidential Business Information
CPID	Consumer Product Information Database
DA	Department of the Army
DESHE	Developmental Environment, Safety, and Occupational Health Evaluation
DGBE	2-(2-Butoxyethoxy)ethanol/ Diethylene glycol monobutyl ether
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
DoE	Department of Energy
DoDI	Department of Defense Instruction
DTIC	Defense Technical Information Center
EC	European Community

EC_{50}	Median (50%) Effect Concentration
ECHA	European Chemicals Agency
ECN	European Community Number
ECOSAR	Ecological Structure Activity Relationship
ECOTOX	USEPA ECOTOXicology Knowledgebase
EPA	U.S. Environmental Protection Agency
EPI Suites™	Estimation Programs Interface (EPI) Suite™
ErC_{50}	Concentration resulting in 50% reduction in growth rate
ESOH	Environment, Safety, and Occupational Health
ESTCP	Environmental Security Technology Certification Program
EOF	Extractable Organic Fluorine
FMS	Flatulent Mouse Syndrome
FY	Fiscal Year
g	Gram
GHS	Globally Harmonized System
HG	2-methyl-2,4-pentanediol
IACUC	Institutional Animal Care and Use Committee
IARC	International Agency for Research on Cancer
Kg	Kilogram
K_H	Henry's Law Constant
LC_{50}	Median (50%) Lethal Concentration
LD_{50}	Median (50%) Lethal Dose
L	Liter

LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
Log K_{OC}	Log organic carbon partition coefficient
Log K_{OW}	Log octanol-water partition coefficient
LTEL	Long-term Exposure Limit
m^3	Cubic meter
µg	Microgram
mg	Milligram
MILSPEC	Military Specification
mL	Milliliter
mm	Millimeter
mmHg	Millimeters (Mm) Of Mercury
MNA	Micronucleus Assay
MW	Molecular Weight
NA	Not Applicable
NCBI	National Center for Biotechnology Information
ND	No Data
NDAA	National Defense Authorization Act
NIOSH	National Institute for Occupational Safety and Health
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level

NRL	Naval Research Lab
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PFAS	Per- and Polyfluoroalkyl Substances
PFOS	Perfluorooctane sulfonic acid
PFOA	Perfluorooctanoic acid
pH	Potential hydrogen
ppb	Parts per billion
PPE	Personal Protective Equipment
ppm	Parts per million
QSAR	Quantitative Structure Activity Relationship
QSM	Quality Systems Manual
RDT&E	Research, Development, Test, and Evaluation
RfC	Reference Concentration
SDS	Safety Data Sheet
SERDP	Strategic Environmental Research and Development Program
SMILES	Simplified Molecular-Input Line-Entry System
SPAC	Single Product Allowable Concentration
STEL	Short-term Exposure Limit
T3	Triiodothyronine
T4	Thyroxine
TAC	Total Allowable Concentration
TOPKAT	Toxicity Prediction by Komputer Assisted Technology

TWA	Time Weighted Average
UPLC-QTOF-MS	Ultra-high Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry
vp	Vapor Pressure
v/v	Volume per volume
WHO	World Health Organization
w/v	Weight per volume

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Toxicology Report No. S.0079790-21 and S.0082073-21-22, June 2022
Toxicology Directorate

Addendum 1: Individual Substance Toxicity Profiles for Toxicology Assessment for Strategic Environmental Research and Development Program: Per- and Polyfluoroalkyl Substances - Free Aqueous Film Forming Foams, July 2020-June 2022

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Specialty: 500c Toxicity

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JULY 2020-June 2022

1. SUMMARY

1.1 Overview

Addendum 1 addresses the current understanding of the toxicity and ecotoxicity of the 21 disclosed constituents of 6 per- and polyfluoroalkyl substances (PFAS) - free aqueous film forming foams (AFFFs). The 21 constituents fall into 5 general classifications: amphiphilic solvents, amphoteric surfactants, anionic surfactants, nonionic surfactants, and carbohydrates (Table 1). Three of the 21 constituents have class or subclass-wide descriptive names (i.e., amphoteric surfactant, alkylbetaine, alkylsulfate). There is also one completely unidentified compound (i.e., “preservative”).

Table 1. Class-Based Grouping of Constituents per Product

	BIOEX® ECOPOL A 3% FFF	Fomtec® Enviro USP	National Foam 20-391	National Foam Avio®F3 Green KHC 3%	NRL 502W (Siloxane-based)	Solberg® Re-Healing™ Foam RF3 3%
Amphoteric Surfactant (+/-)	UNK	308062-28-4	ECN 939-455-3	1643-20-5	67674-67-3	139-96-8
	UNK	90583-18-9		3332-27-2		61789-40-0
						68139-30-0
						96130-61-9
Amphiphilic Solvent (aqueous/organic)	112-34-5	112-34-5	107-41-5	112-34-5	112-34-5	112-34-5
Anionic Surfactant (-)	UNK	68891-38-3	68891-38-3	151-21-3		
Nonionic Surfactant					68515-73-1	68515-73-1
Carbohydrate						57-50-1
						9005-25-8
Unknown	preservative					

Legend:
UNK = unknown
NRL = Naval Research Lab

1.2 Purpose

This appendix is focused on the toxicity of individual constituents identified and interpreted on an individual basis. This division from the main document is intended to increase approachability.

2. REFERENCES

Appendix A lists the references cited in this Addendum. They are organized by the 5 general classifications of the 21 individual constituents.

3. SUMMARIES OF PRODUCTS

3.1 BIOEX ECOPOL A 3% Fluorine Free Foam

BIOEX ECOPOL A 3% Fluorine Free Foam (FFF) is a foam concentrate under consideration as a PFAS-free replacement AFFF by the Department of Defense (DoD). It is a green liquid that foams when agitated. There are five constituents listed in the safety data sheet (SDS) for this product, but only one was described using a Chemical Abstracts Service Registry Number (CASRN), meaning that the four other constituents have not been fully identified or characterized in this toxicity assessment. BIOEX disclosed 10 – 32.5015% of the product, leaving 68.5 – 90% of the product unknown. A best effort has been made to provide relevant toxicity information for the general classes of compounds that comprise this mixture.

3.2 Fomtec Enviro USP

Fomtec Enviro USP is a foam concentrate under consideration as a PFAS-free replacement AFFF by the DoD. It is a light straw-colored viscous liquid (non-Newtonian gel) that foams when agitated. There are four constituents listed in the SDS for this product and all are described using a CASRN. Fomtec disclosed 12 – 23% of the product, leaving 77 – 88% of the product unknown.

3.3 National Foam 20-391

National Foam 20-391 is a foam concentrate under consideration as a PFAS-free replacement AFFF by the DoD. It is a clear non-Newtonian liquid (gel) that foams when agitated. There are three constituents listed in the SDS for this product. Two are described using a CASRN, but one does not have an assigned CASRN. Instead, that component is described with a European Chemical Number (ECN). National Foam disclosed 5 – 24% of the product, leaving 76 – 95% of the product unknown.

3.4 National Foam Avio^{F3} Green KHC 3%

National Foam Avio^{F3} Green KHC 3% is a foam concentrate under consideration as a PFAS-free replacement AFFF by the DoD. It is a light straw-colored liquid that foams when agitated. There are four constituents listed in the SDS for this product and all are described using a CASRN. National Foam disclosed 25 – 61% of the product, leaving 39 – 75% of the product unknown.

3.5 Naval Research Lab 502W Foam Product Concentrate

Naval Research Lab (NRL) 502W Foam Product Concentrate is a foam concentrate under consideration as a PFAS-free replacement AFFF by the DoD. It is a clear amber liquid that foams when agitated. There are three constituents listed in the SDS for this product and all are described using a CASRN. NRL disclosed 33.7% of the product, leaving 66.3% of the product unknown.

3.6 Solberg Re-Healing Foam RF3 3%

Solberg Re-Healing Foam RF3 3% is a foam concentrate under consideration as a PFAS-free replacement AFFF by the DoD. It is an amber-colored viscous liquid (non-Newtonian gel) that foams when agitated. There are eight constituents listed in the SDS for this product and all are described using a CASRN. Solberg disclosed up to 74.5% of the product, leaving 25.5% of the product unknown.

4. SUMMARIES OF INDIVIDUAL CONSTITUENTS

4.1 Amphiphilic Solvents

4.1.1 Diethylene Glycol Monobutyl Ether (DGBE) [CSARN 112-34-5]

DGBE [CSARN 112-34-5], also identified as 2-(2-butoxyethoxy)ethanol and butyl carbitol, belongs to the glycol ether subclass of amphiphilic surfactants. DGBE is of low toxicity by all routes of exposure with the exception of the eyes. It is not expected to be a developmental or reproductive toxicant, genotoxic, or carcinogenic. Ecotoxicity is low, and DGBE will be readily degraded in the environment. DGBE is found in BIOEX ECOPOL A 3% at 10–25%, Fomtec ENVIRO USP at 5–10%, National Foam AvioF3 Green KHC 3% at 10–30%, NRL 502W at 17%, and Solberg Re-Healing Foam RF3 3% at <20%.

4.1.2 Hexylene Glycol (HG) [CASRN 107-41-5]

HG [CASRN 107-41-5], also identified as 2-methyl-2,4-pentanediol, belongs to the diol subclass of amphiphilic surfactants. HG is of low toxicity by all routes of exposure with the exception of the eyes, where it is of moderate toxicity but effects are reversible. It is not expected to be a developmental or reproductive toxicant, or be genotoxic. Due to lack of data, this substance has not been classified as a carcinogen. Ecotoxicity of HG is low, and it is not expected to bioaccumulate and is considered to be at least inherently biodegradable. However, it is highly water soluble and will potentially move large distances in aquatic systems, so exposure in aquatic systems is likely. HG is present in National Foam 20-391 at 4 – 10%.

4.2 Amphoteric Surfactants

An unidentified amphoteric surfactant is present in BIOEX ECOPOL A 3%. For a general description of this class, see paragraph 7.2.2 in the main document.

4.2.1 Alkyl Hydroxysultaines

4.2.1.1 1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,Ndimethyl-3-sulfo-, N-coco Acyl Derivs., Hydroxides, Inner Salts (CAPHS C12) [CASRN 68139-30-0]

CAPHS C12 [CASRN 68139-30-0] belongs to the alkyl hydroxysultaines subclass of amphoteric surfactants. CAPHS C12 is not acutely toxic in mammals, fish, daphnia, or algae. Additionally, it has low hazard potential as a reproductive/developmental toxicant and moderate hazard potential subchronically. CAPHS C12 has low bioaccumulation and rapidly biodegrades. As such, primary concerns associated with this chemical is the potential for skin and eye irritation in mammals. CAPHS C22 is found in Solberg Re-Healing Foam RF3 3% at <2.5%.

4.2.1.2 1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-(C8-18(even numbered) acyl) Derivs., Hydroxides, Inner Salts (CAPHC C8-18) [ECN: 939-455-3]

CAPHS C8-18 [ECN: 939-455-3] belongs to the alkyl hydroxysultaines subclass of amphoteric surfactants. Using the toxicological data for a twelve carbon chain CAPHS, the following recommendations and conclusions can be presumed for CAPHS C8-18. This substance is Category 5 for oral acute toxicity based on GHS criteria: it is not a dermal sensitizer, dermal irritant, or genotoxic. Inhalation exposure is expected to be minimal, but ocular exposure is proven to be moderate to severely irritating. This substance decomposes rapidly in the environment and is considered nonvolatile. This substance is of low to moderate toxicity to aquatic species. CAPHS C8-18 is found in National Foam 20-391 at 4 – 10%.

4.2.2 Alkylbetaines

An unidentified alkylbetaine, a subclass of amphoteric surfactant, is a component of BIOEX ECOPOL A 3% FFF at 0 – 2.5%. The biggest areas of concern are likely moderate oral toxicity and high dermal and ocular toxicity based on information in the SDS. It is unknown how the release of this class of compounds into the environment will impact the ecosystem.

4.2.3 Alpha-sulfo-omega-hydroxy-poly(oxy-1,2-ethanediyl)C9-11 Alkyl Ethers, Sodium Salts (AES C9-11 1-3EO Na) [CASRN 96130-61-9]

AES C9-11 1-3EO Na [CASRN 96130-61-9] belongs to the alkylethoxy sulfate subclass of amphoteric surfactants. In general, AES C9-11 1-3EO Na appears to be of low toxicity in human relevant models and presents main hazards associated with skin and eye irritation following high concentration exposure. Reducing concentrations can help mitigate this irritation. Environmental toxicity to aquatic systems post wastewater treatment appear to largely be focused on algal impacts, but is highly influenced by the short half-life in both aerobic and anaerobic systems. AES C9-11 1-3EO Na is found in Solberg Re-Healing Foam RF3 3% at <5%.

4.2.4 1-propanaminium, 3-amino-N-carboxymethyl) -N,N-dimethyl-,N-coco acyl derivs., Hydroxides, Inner Salts (CAPB) [CASRN 61789-40-0]

CAPB [CASRN 61789-40-0] belongs to the amidoproyl betaine subclass of amphoteric surfactants. CAPB is a skin and eye irritant in humans and mammals, but is not acutely toxic at 5,000 milligrams per kilogram (mg/kg). There is a potential for chronic effects of exposure based on observed gut absorption and detectable levels of CAPB in the urinary system.

CAPB has a predicted high acute aquatic toxicity in fish, aquatic invertebrates, and aquatic plants. CAPB partitions to soil and water, has a low bioconcentration factor (BCF), and is readily biodegradable. Therefore the highest risks to aquatic organisms are likely acute, but there are no empirical data to support model predictions. CAPB is found in Solberg Re-Healing Foam RF3 3% at <20%.

4.2.5 Amine Oxides

4.2.5.1 Lauramine Oxide (AO C12) [CASRN 1643-20-5]

AO C12 [CASRN 1643-20-5] belongs to the amine oxide subclass of amphoteric surfactants. Concerns with AO C12 are related to its irritation and corrosive properties at high concentrations and aquatic toxicity. It is found in National Foam Avio^{F3} Green KHC 3% at 7 – 13%.

4.2.5.2 Dimethyltetradecylamine Oxide (AO C14) [CASRN 3332-27-2]

AO C14 [CASRN 3332-27-2] belongs to the amine oxide subclass of amphoteric surfactants. The largest concerns are associated with irritation and corrosive impacts of dermal or ocular exposure to high concentration AO C14. As the product is normally produced and distributed in low concentrations in aqueous solution, toxic effects through other oral or inhalation exposure routes are unlikely to occur. If exposure does occur, effects are observed at relatively high doses. Environmental concerns are associated with high acute and chronic toxicity but a short half-life. Accordingly, reducing direct releases to aquatic environments are suggested but in normal waste disposal streams, impacts are likely to be low. AO C14 is found in National Foam Avio^{F3} Green KHC 3% at 1 – 5%.

4.2.5.3 Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides (AO C12-14) [CASRN 308062-28-4]

AO C12-14 [CASRN 308062-28-4] belongs to the amine oxide subclass of amphoteric surfactants. AO C12-14 causes skin irritation, is harmful if swallowed, and causes serious eye damage. It is toxic to aquatic life with long lasting effects. AO C12-14 is found in Fomtec ENVIRO USP at <1%.

4.2.6 502W Additive (PDMS EO) [CASRN 67674-67-3]

PDMS EO [CASRN 67674-67-3] belongs to the ethoxylated siloxane subclass of amphoteric surfactants. According to the classification provided by companies to European Chemical Agency (ECHA), this substance is toxic to aquatic life with long lasting effects, causing serious

eye damage and is harmful if inhaled. PDMS EO is found in NRL 502W (Siloxane-based) at 6.7%. More information on this siloxane can be obtained in a report from NRL (NRL 2021).

4.2.7 Organosubstituted Sulfates

4.2.7.1 Tris(2-hydroxyethyl)ammonium Dodecylsulfate (AS C12 TEA) [CASRN 139-96-8]

AS C12 TEA [CASRN 139-96-8] belongs to the organosubstituted sulfate subclass of amphoteric surfactants. AS C12 TEA toxicity data are heavily influenced by its performance in acute limit tests (e.g., no toxicity at the limit test). The vast body of literature and wide acceptance of read-across to other cations (Na, in particular) and other C-chain lengths (12, 12-14, etc.) reduces the amount of compound-specific data. Toxicity and risk of alkyl sulfates, as a class, is largely based on C-chain determinant toxicity regardless of cation.

Otherwise, acute toxicity is low, chronic toxicity is low, and the major sources of concern are dermal and ocular exposure and resultant irritation at high concentrations. Aquatic toxicity is likely another area of concern, but that is largely based on modeled data and read-across from alkyl sulfates as a class. Importantly, there is confidence that aquatic toxicity will likely be impacted by a short biodegradation half-life. AS C12 TEA is found in Solberg Re-healing Foam RF3 3% at <20%.

4.2.7.2 Sulfuric Acid, Mono-C12-14-alkyl Esters, Compounds with Triethanolamine (AS C12-14 TEA) [CASRN 90583-18-9]

AS C12-14 TEA [CASRN 90583-18-9] belongs to the organosubstituted sulfate subclass of amphoteric surfactants. AS C12-14 TEA has a moderate amount of data, but as a member of the alkyl sulfates class of anionic surfactants, has a vast body of information with potential for read-across. Overall, acute toxicity is low to moderate, chronic toxicity is low, and the major sources of concern are associated with dermal or ocular exposure to high concentrations and aquatic toxicity. AS C12-14 TEA is a severe irritant to dermal and ocular tissues at high concentration, though this is dose-dependent, and at cosmetic levels, irritation is limited. In aquatic systems, though biodegradation is fast, effects in algae and invertebrates can occur acutely. Importantly, experimental data in aquatic systems shows less toxicity than model predictions. AS C12-14 TEA is found in Fomtec ENVIRO USP at 5 – 9%.

4.3 Anionic Surfactants

4.3.1 Alkyl Sulfates

An unidentified alkyl sulfate, a subclass of anionic surfactant, is present in BIOEX ECOPOL A 3%. As a subclass, alkyl sulfates are low-to-moderately toxic via the oral route. Alkyl sulfates have moderate dermal toxicity and high ocular toxicity.

4.3.1.1 Sodium Lauryl Sulfate (AS C12 Na) [CASRN 151-21-3]

AS C12 Na [CASRN 151-21-3] belongs to the alkyl sulfate subclass of anionic surfactants. AS C12 Na has moderate oral toxicity and high dermal and ocular toxicity. AS C12 Na has

moderate toxicity in aquatic systems. It is found in National Foam Avio^{F3} Green KHC 3% at 7 – 13%.

4.3.2 Sodium Laureth Sulfate (AES C12-14 2.5EO Na) [CASRN 68891-38-3]

AES C12-14 2.5EO Na [CASRN 68891-38-3] belongs to the alkylethoxy sulfate subclass of anionic surfactants. AES C12-14 2.5EO Na represents a generic surfactant (similar to CASRN 9004-82-4) of low toxicity. Irritation appears to be the dominant source of toxicological hazards to human exposure and this can be mitigated by rinsing and reducing concentrations. Environmental effect potential is high due to large usage and low LC50s/HC5s, but biodegradation rates are high and wastewater treatment activities highly effective. AES C12-14 2.5EO Na is found in Fomtec ENVIRO USP at 1 – 3% and National Foam 20-391 at 1 – 4%.

4.4 Carbohydrates

4.4.1 Sucrose [CASRN 57-50-1]

Sucrose [CASRN 57-50-1] is a carbohydrate. Sucrose is deemed Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA). It is found in Solberg Re-healing Foam RF3 3% at >1%.

4.4.2 Starch [CASRN 9005-25-8]

Starch [CASRN 9005-25-8] is a carbohydrate. Starch is deemed GRAS by the FDA and is exempt from U.S. Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA) reporting. It is found in Solberg Re-healing Foam RF3 3% at >1%.

4.5 Nonionic Surfactants

4.5.1 Glucopon® 225DK (DG) [CASRN 68515-73-1]

Glucopon 225DK or decyl glucoside (DG) [CASRN 68515-73-1] belongs to the alkyl glucoside subclass of nonionic surfactants. The main hazards for DG are skin and eye irritation. DG is considered a skin irritant with allergenic and sensitization properties, and exposure can cause serious eye damage and irritation. As an alkyl glucoside, DG may increase permeability of the skin thereby allowing other compounds in a product that otherwise are not dermally absorbed to penetrate the dermal barrier, potentially changing the hazards associated with those compounds within the product. DG is listed as practically nontoxic via the oral route, but can be fatal if it is aspirated into the airway while being swallowed. No mutagenicity was observed for DG, and it is likely not a carcinogen or reproductive/developmental toxicant.

Although DG is considered a moderate hazard for both acute and chronic aquatic toxicity, it is also listed as a green circle chemical within the EPA Safer Chemical Ingredients List (SCIL), making it a chemical of low environmental concern. DG is readily biodegradable, and is therefore not a bioaccumulation or environmental persistence hazard. DG is found in NRL 502W (Siloxane-based) at 10% and in Solberg Re-healing Foam RF3 3% at <5%. More information on this alkylpolyglycoside can be obtained in reports from NRL (NRL 2021, NRL 2019).

4.6 Unidentified

4.6.1 Preservative [CASRN not available]

The specific ingredient is not disclosed by the manufacturer.

5. SUBSTANCE TOXICITY PROFILES

5.1 Amphiphilic Solvents

5.1.1 Diethylene glycol monobutyl ether (DGBE) [CSARN 112-34-5]

2-(2-butoxyethoxy)-ethanol, also known as diethylene glycol monobutyl ether (DGBE) is a colorless liquid with a mild odor (NCBI 2020a). It is the primary glycol ether solvent in most AFFFs. It is used as an inert ingredient that is not registered for current use as a pesticide in the United States, but is used in pesticide products. It is also used as a coalescing agent in latex paints, solvent for stamp pad inks, dye solvent, solvent in high baked enamels, dispersant, diluent for hydraulic brake fluids, and a mutual solvent for soap, oil, and water in household cleaners (NCBI 2020a).Figure 1 shows the structure of DGBE.

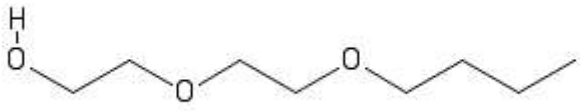

Figure 1. Structure of DGBE (PubChem Sketcher 2021)

5.1.1.1 Toxicology Data

5.1.1.1.1 Oral

In three acute oral studies in rats, median lethal dose (LD50) values were 6,560, 7,291, and 9,623 milligrams per kilogram (mg/kg) body weight. In two acute oral studies in mice, LD50 values ranged from 2,410 to 5,530 mg/kg. Finally, the acute oral LD50 in guinea pigs is reported as 2,000 mg/kg (CompTox 2020a). The acute oral LD50 in rats is reported to be 5,660 mg/kg (BIOVIA 2015). TOPKAT modeling predicts a lowest observed adverse effect level (LOAEL) of 1,500 milligram per kilogram body weight per day (mg/kg-d) at high confidence. These values classify DGBE in the low toxicity category, and Category 5 according to the Global Harmonized System (GHS) categories.

No pertinent data regarding human, or chronic animal oral exposure were located (EPA 2009). Mild liver, kidney, testicular, spleen, and blood effects were observed in laboratory animals repeatedly exposed to high-to-very-high oral doses of DGBE (NCBI 2020a).

Specifically, one subchronic 5 days/week, 6-week study by Kodak et al. (1984), exposed rats via oral gavage to 891, 1,782, or 3,564 mg/kg-d DGBE. The endpoints included clinical signs, food consumption, body weight, hematology, clinical chemistry, organ weights, gross pathology, and histopathology. The critical effects observed were hyperkeratosis of the stomach at ≥ 891 mg/kg-d, and hematologic effects which included reduced erythrocyte count, hemoglobin concentration, and mean corpuscular hemoglobin concentration (MCHC), and increased mean corpuscular volume (MCV) at ≥ 1,782 mg/kg-d (Kodak 1984). Another subchronic 5 days/week, 13-week study by Hobson et al. (1987), exposed rats via oral gavage to 0, 70, 330, or 1,630 mg/kg-d (males) or 0, 50, 250, 1,270 mg/kg-d (females). The critical effects observed were decreased total white blood cell (WBC) and lymphocyte counts and MCHC in females at ≥ 50 mg/kg-d. A LOAEL of 50 mg/kg-d was established at which there was lymphopenia (Hobson et al. 1987).

In a subchronic drinking water study, rats were exposed for 7 days/week for 13 weeks to 0, 50, 250, or 1,000 mg/kg-d DGBE. The endpoints included clinical signs, food and water consumption, body weight, hematology, clinical chemistry, urinalysis, functional observational battery (FOB), sperm analysis, liver metabolic enzymes, organ weights, gross pathology, and histopathology. Critical effects observed were decreased red blood cell (RBC) count, hemoglobin, and hematocrit at ≥ 250 mg/kg-d. Other effects only occurred at 1,000 mg/kg-d and included increases in organ weight and hepatic cytochrome P450s and UDP-glucuronosyltransferase (UGT) levels, decreases in serum total protein, cholesterol, and serum aspartate aminotransferase (AST), and hepatocyte hypertrophy and individual hepatocyte degeneration. They established a no observed adverse effect level (NOAEL) of 50 mg/kg-d, and LOAEL of 250 mg/kg-d at which there was reduced RBC count and hemoglobin (Hgb) in both sexes (Johnson et al. 2005).

5.1.1.1.2 Inhalation

TOPKAT modeling predicts an acute median lethal concentration (LC50) in rats of more than 10 grams per meter cubed per hour (g/m^3-hour).

No pertinent data regarding human, or chronic animal inhalation exposure were located (EPA 2009).

In a 6 hours/day, 5 days/week for 5 weeks subchronic study by Gushow et al., rats received 0, 2, 6, or 18 parts per million (ppm) DGBE. The endpoints included clinical signs, body weight, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, and histopathology. The critical effects observed were slight hepatocyte vacuolization consistent with fatty change in females at ≥6 ppm and gross paleness of the liver in 3/10 females at 18 ppm. A NOAEL of 2 ppm, and LOAEL of 6 ppm was established (Gushow et al. 1984).

5.1.1.1.3 Dermal

Animal studies do not indicate DGBE causes allergic skin reactions, but it is classified as an irritant (NCBI 2020a).

5.1.1.1.4 Ocular

DGBE has been shown in animal studies to be an ocular irritant, and is classified by ECHA as extremely irritating to eyes (NCBI 2020a).

In a rabbit study using instillation volumes of 0.1 milliliter (mL), undiluted DGBE caused moderately severe conjunctivitis, with mild blepharitis, and just detectable to mild diffuse keratitis. The first 24 hours post instillation were the most marked, with effects subsiding and no residual tissue injury by 14 days. A NOAEL of 5% volume per volume (v/v) was established, with no local inflammation occurring, and a LOAEL of 25% v/v, producing just detectable keratitis (Ballantyne 1984).

In an acute dermal study with guinea pigs the LD50 was 2 milliliters per kilogram body weight (mL/kg), and in an acute dermal study with rabbits the LD50 was 2,764 mg/kg (CompTox 2020a).

5.1.1.1.5 Development and Reproduction

There are no indications that reproductive or developmental toxicity are effects of concern for DGBE. Multiple oral studies in rats and mice found no effects at doses of 500, 633 mg/kg-d (EPA 2009). Only one study had a slight reduction in pup weight during the last week of lactation in the offspring of the females dosed with 1,000 mg/kg-d. No maternal toxicity or reproductive effects were observed (Nolen et al. 1985). In another study, rats were exposed to 1000 mg/kg-d DGBE for 9 – 10 weeks. Fertility and viability were unaffected in offspring (Sitarek 2012). Dermal studies also showed no effects on reproduction or development (EPA 2009).

5.1.1.1.6 Genotoxicity

The genotoxicity of DGBE has been tested in *in vitro* assays with bacteria and mammalian cells, and *in vivo* testing with fruit flies and mice. DGBE was negative for reverse mutation in *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation (Thompson et al. 1984; Zeiger et al. 1992), forward mutation in Chinese hamster ovary (CHO) cells (Gollapudi et al. 1993), and sister chromatid exchanges (SCEs) in CHO cells. *In vivo* testing of DGBE was negative for sex-linked recessive mutations in *Drosophila melanogaster* (Thompson et al. 1984) and induction of micronuclei in bone marrow cells of mice (Gollapudi et al. 1993). In one study with mouse lymphoma L5178Y cells, DGBE tested weakly positive for forward mutations (Thompson et al. 1984).

5.1.1.1.7 Carcinogenicity

DGBE has not been classified as a carcinogen due to lack of data. TOPKAT modeling predicts DGBE will not be carcinogenic.

5.1.1.1.8 Neurotoxicity

There are no indications that neurotoxicity is concern for DGBE. An oral study in rats found no behavioral effects at doses of 50, 250, or 1,000 mg/kg-d (Johnson 2005). Similarly, a dermal study in rats showed no effects on a number of neurotoxicity endpoints (EPA 2009).

5.1.1.1.9 Mode/Mechanism of Action

No data regarding mechanism of action were found, but the majority of high dose oral exposures show a pattern of hematological effects as systemic toxicity endpoints (EPA 2009).

5.1.1.2 Ecological Data

5.1.1.2.1 Fate and Transport

Distribution modeling suggests that environmental concentrations of DGBE are likely to be low, and if released into the environment, it will be broken down in air, but may not be broken down by light. Any DGBE present in the atmosphere is expected to exist almost entirely in the vapor phase. It is not expected to absorb ultraviolet (UV) light in the environmentally significant range of >290 nanometers (nm); therefore, is not anticipated to undergo direct photolysis either on sunlit soil surfaces or in aquatic environments (ECHA 2011). Although it is unlikely to move into the air from soil and water surfaces due to a very low Henry's Law constant (K_H), it is estimated that DGBE will move easily through soil based on its organic carbon partition coefficient (K_{OC}) of 10. DGBE will also readily evaporate from dry surfaces due to its vapor pressure. No experimental data on bioaccumulation was found, but we can expect DGBE to have a low bioaccumulation potential in the environment due to its very low modeled log octanol-water partition (K_{OW}), 0.56 (NCBI 2020a). Based on a K_{OC} of 3.6 liters per kilogram body weight (L/kg), it's expected to be highly mobile in soil (Bodar 2008). It is generally classified by the EPA as "practically nontoxic" to aquatic organisms based on acute toxicity, and conservatively calculated exposures are mostly below concentrations of concern for chronic risks to aquatic life (Staples et al. 1998). An estimated BCF of 3 also suggests the potential for bioconcentration in aquatic organisms is low (NCBI 2020a).

5.1.1.2.2 Ecotoxicity

A number of studies show that DGBE is practically acutely nontoxic to fish (LC50 value = 1,300 mg/L). A median effective concentration (EC50) value of >1,100 mg/L was determined for invertebrates using a number of studies and quantitative structure activity relationship (QSAR) modeling. QSAR modeling also predicts that algae is likely to be the most sensitive species, with LD50 values of 3,978, 1,742, and 394 mg/L in fish, invertebrates, and algae, respectively. There is no experimental chronic invertebrate toxicity data available, but QSAR predictions show that DGBE is of low toxicity at chronic levels of exposure (ECHA 2011). LC50 values in a type of green algae, *Desmodesmus subspicatus*, were >100 mg/L for >96 hours. LC50 values for *Lepomis macrochirus* (bluegill) in a static exposure were 1,300 mg/L at 96 hours,

5.1.1.2.3 Degradation and Treatment

DGBE is predicted to be readily biodegradable with environmental persistence of only days (Bodar 2008), and a predicted average half-life being 3.68 days (CompTox 2020a). In one study, DGBE degraded 14, 19, 60 and 100% when incubated for 1, 3, 5, and 6 days, respectively, using a nonadapted activated sludge and a modified Zahn-Wellens test. In the same report, another modified Zahn-Wellens test using nonadapted activated sludge gave 100% degradation after 9 days, and a test using adapted activated sludge showed 58 and >60% removal after 28 days (Staples et al 1998). In another study, DGBE, present at 100 mg/L reached 92% of its theoretical biochemical oxygen demand (BOD) in 4 weeks using an activated sludge inoculum at 30 mg/L in the Japanese Ministry of International Trade and Indsitry (MITI) test (National Institution of Technology and Evaluation (NITE) 2015).

DGBE will not be readily removed from waste streams by physical processes at wastewater treatment plants (WWTPs), but should be readily treated by biodegradation.

5.1.2 Hexylene glycol (HG) [CASRN 107-41-5]

2-Methyl-2,4-pentanediol, also known as hexylene glycol (HG), is an oily colorless liquid with a mild sweet odor (NCBI 2020b). It occurs naturally as an aroma and flavor component of Red Delicious apples, and as a component in a large number of products for industrial and consumer use (Screening Information Data Sets (SIDS) 2001). It is used in lubricants and greases, adhesives and sealants, polishes and waxes, anti-freeze products, cosmetics and personal care products, hair care products, washing and cleaning products, coating products, fillers, putties, plasters, modelling clay and finger paints. Industrial coatings account for about 45% of the total production. Data indicate that the general population may be exposed to HG via ingestion of apples, but mainly through its use in cosmetics, antifreezes, and hydraulic fluids (NCBI 2020b, SIDS 2001). Indirect exposures via the environment such as via ingestion of surface water contaminated with HG are also possible (SIDS 2001). Figure 2 shows the structure of HG.

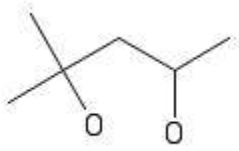

Figure 1. Structure of 2-methyl-2,4-pentanediol (HG) (PubChem Sketcher 2021)

5.1.2.1 Toxicology Data

5.1.2.1.1 Oral

In a human exposure study, five subjects were given oral doses of 37 g HG daily for 24 days (Jacobson 1958). The estimated daily dose was 14 – 28 mg/kg, and no subjective symptoms or alterations in urine parameters were detected.

In an acute mouse study, hypoactivity occurred following single doses of 1.85 g/kg. HG caused irritation of the lungs and large intestine, but no gross effects were apparent in the brain, kidney, or heart (NCBI 2020b). In an acute rat study, a NOAEL of 2,000 mg/kg was established (ECHA 2020a; Gardner 1996a). Overall, the acute oral LD50 for mammals is >2,000 – 4,700 mg/kg (SIDS 2001). According to oral GHS acute toxicity, HG is categorized as GHS category 5.

In a 90-day study, Sprague Dawley rats were exposed via oral gavage to 0, 50, 150, and 450 mg/kg-d HG (Fabreguettes 1999). A FOB gave no evidence of neurotoxic effects. Increased liver weight and hepatocellular hypertrophy were observed at 450 mg/kg-d in both sexes, and in males only at 150 mg/kg-d. These changes were considered an adaptive response to increased metabolic demand since no degenerative or necrotic changes were observed. Increased kidney weights and higher incidence and severity of acidophilic globules in the tubular epithelium were observed in male rats at 150 and 450 mg/kg-d. No adverse effects were found in other organs, including the reproductive organs. The systemic NOAEL was determined to be 450 mg/kg-d, and a NOAEL for localized irritation of the gastrointestinal (GI) tract was 50 mg/kg-d (Fabreguettes 1999).

In one study, male rats were given 0, 100, or 200 mg HG in milk for 129 days (calculated average daily intake of 148 – 190 mg/kg-d due to resistance to consume) (Larsen 1958). All liver tissues and testes were normal, and 7 out of 10 kidneys were normal in the high dosed group. No pathological signs of toxicity were observed, including behavioral.

5.1.2.1.2 Inhalation

Human volunteers were exposed to saturated room concentrations at 50 ppm HG for 15 minutes (Silverman et al. 1946). An average number of 12 subjects of both sexes were used for each solvent exposure. The maximum tolerable concentration was considered to be 50 ppm, where most people had eye irritation but no irritation to the nose or throat. In a separate study, some volunteers reported slight nasal irritation and respiratory discomfort with eye irritation following exposure to 100 ppm HG for 5 minutes (Hine et al. 1955).

Inhalation exposure of saturated HG vapor to laboratory animals at room temperature (60 ppm) or vapor heated (18,000 ppm) did not produce acute intoxication or lethality (Smyth and Carpenter 1948). Accordingly, a threshold limit value (TLV) of 25 ppm as a ceiling value is recommended in order to avoid eye irritation from HG (ACGIH 2015). Inhalation exposure is less of a concern considering the low vapor pressure of HG (SIDS 2001).

5.1.2.1.3 Dermal

Studies of groups of 37 and 39 human subjects with healthy skin also demonstrated that HG is not an irritant. Irritation scores were 0.11 for a 24-hour occluded patch test and 0.02 for a semi-occluded patch, when rated on a scale of 0 to 4 (Cosmetic Ingredient Review (CIR) 1985). Finally, a study was conducted using 823 eczema patients in a 48-hour occlusive patch test at aqueous concentrations of 30% or 50% HG (Kinnunen and Hannuksela 1989). These concentrations caused edema and erythema of the skin in 2.8% of the patients.

In a study carried out to Organisation for Economic Co-Operation and Development (OECD) Test No. 404, 0.5 mL pure HG was applied to the skin of rabbits in a 4-hour semi-occlusive exposure (Parcell 1995). Group mean (24+48+72 hour) scores were 0.4 for erythema and 0 for edema, leading to the conclusion that HG is not a skin irritant under conditions of the study. In studies carried out under cosmetic guidelines using both 24-hour and repeated exposure, low level of irritation potential was found (Guillot et al. 1982). In an OECD Test No. 402 acute dermal toxicity study in rats, no irritation was observed following a 24-hour covered application of 2,000 mg/kg undiluted HG (Gardner 1996b).

The dermal LD50 is >2,000 mg/kg (range >1.84 – 12.3 g/kg) (SIDS 2001). Accordingly, based on GHS acute toxicity classification, HG is category 5.

5.1.2.1.4 Ocular

In a study carried out to OECD Test No. 405, undiluted HG was found to be slightly irritating to the eye (Gardner 1996c). Group mean (24+48+72 hour) scores were corneal opacity 0.8, iritis 0, conjunctival redness 0.9, and chemosis 0.9. Maximum individual (24+48+72 hour) scores were corneal opacity 1 (observed in 1 rabbit), iritis 0, conjunctival redness 1, and chemosis 1.3. All signs of irritation were reversible. Studies carried out under national cosmetic guidelines indicated that undiluted HG caused initial irritation, which was reversible within 7 days (Guillot et al. 1982).

In an acute exposure experiment, the undiluted material was introduced into the eyes of rabbits where it caused considerable irritation and corneal injury that was slow to heal (Bingham et al. 2001).

According to GHS eye effects classification, HG is a category 2 eye irritant, subcategory B, mild irritant, reversible in 7 days.

5.1.2.1.5 Development and Reproduction

In a subacute developmental toxicity study, 24 female rats were exposed via oral gavage to 30, 300, or 1,000 mg/kg-d HG (SIDS 2001, NCBI 2020b). A NOAEL for maternal toxicity of 300 mg/kg-d was based on a reduction in group mean body weight gain on gestation day (GD) 6–7 (ECHA 2020a, SIDS 2001). The LOAEL for maternal toxicity was 1,000 mg/kg-d based on reduced body weight gain. This dose was also the LOAEL for fetal toxicity based on a slight delay in ossification, a greater number of fetuses with extra thoraco-lumbar ribs, and a slight decrease in fetal body weight (not statistically significant).

In the rat study previously described, males were given 148 – 190 mg/kg-d HG. After 87 days, six control and seven treated males were mated with up to seven different untreated females over a 47-day period (SIDS 2001). The animals were paired until pregnancy was confirmed via vaginal smear. Pregnancy was terminated 1 – 2 days prior to the estimated delivery and the number of live fetuses were counted. There were no statistically significant differences between treated and control groups. Limited conclusions can be drawn from this study, as the methods were invalid and no statistical analysis was performed.

In a 90-day oral study where HG was administered at doses up to 450 mg/kg-d, no effects on the gonads were observed (SIDS 2001). A subacute oral toxicity study derived a NOAEL of 500 mg/kg-d for rats with an effect on fertility (ECHA 2020a).

5.1.2.1.6 Genotoxicity

Hexylene glycol is not genotoxic in mammalian or nonmammalian cells *in vitro* (SIDS 2001). In an Ames test, HG produced negative test results for *S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98, TA 100, and *Escherichia coli* WP2 uvr A pKM 101 with and without S9 activation. No increased incidence in reverse mutation rates were observed. A mitotic recombination test in *Saccharomyces cerevisiae* produced negative results with and without metabolic activation. Finally, a chromosomal aberration test on CHO cells produced negative results with and without metabolic activation.

5.1.2.1.7 Carcinogenicity

HG has not been classified as a carcinogen due to lack of data.

5.1.2.1.8 Neurotoxicity

There are no indications that HG is neurotoxic. A subchronic oral study that included a FOB gave no evidence of neurotoxic effects (Fabreguettes 1999).

5.1.2.1.9 Mode/Mechanism of Action

HG was tested in 858 bioassays and reported as active in 22 (CompTox 2020b). Eight bioassays were aimed at targeting the cell cycle and their lowest observed effect concentrations (LOECs) ranged from 0.300 to 83.7 micromolar (µM), with the median being 2.225 µM. There were 7 assays intended to target DNA binding with LOECs ranging from 0.0971 to 0.183 µM, and a median of 0.0519 µM. The median LOEC values for 2 nuclear receptor and 2 G protein-coupled receptor (GPCR) assays were 31.1 and 0.07305 µM, respectively. The LOEC values for cell morphology, cytokine, and cytokine receptor were 1.72, 0.30, and 40.8 µM, respectively.

No clear mechanism of action has been identified for HG.

5.1.2.2 Ecological Data

5.1.2.2.1 Fate and Transport

HG is predicted to distribute in the environment primarily to water or water and soil. It has a high water solubility of 68,780 mg/L, and a log K_{oc} of <1 (European Union (EU) Technical Guidance Document (TGD) QSAR 1996). If released into water, HG is not expected to adsorb to suspended solids and sediment based on an estimated BCF of 3.162 and K_{ow} of 0.58 (CompTox 2020b, SIDS 2001). With a vapor pressure of 0.07 millimeters of mercury (mmHg), HG will exist solely as a vapor in the atmosphere if released into air (NCBI 2020b). The calculated half-life for the photo-oxidation of HG in air is 9 hours, it is not expected to undergo direct photolysis, and is not susceptible to hydrolysis (SIDS 2001).

Normal manufacturing practices should not emit HG into the atmosphere, but low levels of emissions may occur as a result of spills and cleaning operations (SIDS 2001). There are no aqueous streams from the production process, but small amounts of HG will be present in the output to the WWTP from spills and cleaning operations. HG can also enter the aqueous and terrestrial environment from end uses such as in agricultural products and down-hole lubricants for oil and gas fields (SIDS 2001).

5.1.2.2.2 Ecotoxicity

Various studies have been conducted with fish and invertebrate species, indicating that HG is of low acute toxicity to aquatic organisms (SIDS 2001). The LC50 values for *Daphnia magna* range from 3,200 mg/L to 5,410 mg/L (Elnabarawy et al. 1986, Thurston et al. 1985). The lowest valid 96-hour LC50 for fish was 8,510 mg/L for *Gambusia affinis,* mosquito fish (Thurston et al. 1985), and the lowest valid 48-hour EC50 for invertebrates was 2,800 mg/L for *Ceriodaphnia reticulata,* water flea (Elnabarawy et al. 1986). Tadpoles of the American bullfrog, *Rana catesbiana* were tested, with a 96-hour EC50 of 11,800 mg/L (Thurston et al. 1985).

The predicted no-effect concentration (PNEC) values for freshwater and marine water organisms are 429 micrograms per liter (µg/L) and 42.9 µg/L, respectively. The derived PNEC value for terrestrial organisms is between 0.066 mg/kg (ECHA 2020a) and 0.0786 mg/kg for soil, and 0.295 mg/kg for sediment (SIDS 2001). An EC_{10} of 200 mg/L was derived for microorganisms. An EC_{10} of 429 mg/L was derived for freshwater algae and cyanobacteria. An EC50 of 2.8 g/L was derived for freshwater invertebrates. An LC50 of 8.51 g/L was derived for freshwater fish (ECHA 2020a, SIDS 2001).

5.1.2.2.3 Degradation and Treatment

HG is considered to be inherently biodegradable (SIDS 2001). The percentage of ready biodegradability assays that passed were 14%, 17%, 60%, and 69% for MITI I (n=7), closed bottle (n=6), sturm nonadapted (n=5), and modified OECD (n=16), respectively. Two tests for inherent biodegradability were included in the round-robin, the Zahn-Wellens test (n=5) and a MITI II assay (n=8), with pass rates of 100% and 50%, respectively (Blok et al. 1985).

5.2 Amphoteric Surfactants

5.2.1 Unidentified amphoteric surfactant [CASRN not available]

Amphoteric surfactants have both cations, anions, and depending on the pH of the solution, may also be zwitterionic (simultaneous negative and positive charges (See Figure 3)) (Ivankovic and Hrenovic 2010). To illustrate amphoteric surfactants as a class, this section will use amine oxide (AO), which is used in foam boosters, anti-static agents, foam stabilizers, polymerization catalysts, and antibacterial agents (Ivankovic and Hrenovic 2010).

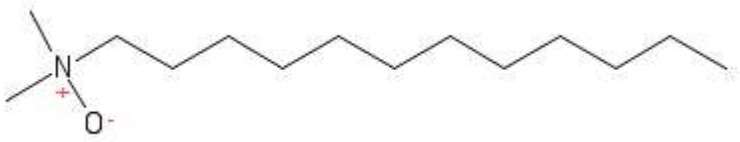

Figure 2. An Amine Oxide is provided as a Representative Amphoteric Surfactant with both a Positive and Negative Charge (PubChem Sketcher 2021)

5.2.1.1 Toxicology Data

5.2.1.1.1 Oral

Rat acute toxicity via gavage is 600 mg AO C10-16/kg. In a 90-day repeated dose test in rabbits exposed to AO in diet, no treatment-related changes were observed in clinical chemistry, hematology, or histopathology, but there were effects including lower body weight gain, lenticular opacities, and diarrhea. The NOAEL for AO is 80 mg/kg-d and the LOAEL is 87 – 150 mg/kg-d (SIDS 2006).

5.2.1.1.2 Inhalation

In an acute inhalation study where rats were exposed to aerosol droplets of 0.016 mg/L as AO in a mixture, no deaths were observed (SIDS 2006).

5.2.1.1.3 Dermal

An acute dermal toxicity test at 520 mg AO/kg (2 mL/kg of a 30% product) resulted in no deaths. Three dermal repeated-dose tests at up to 1.5 mg AO/kg-day resulted in no dermal irritation. There are no reports of skin sensitization due to AO exposure (SIDS 2006).

5.2.1.1.4 Ocular

Amine oxides of various carbon chain lengths are not irritating to the eyes at 1%, moderately irritating at 5%, and severely irritating at 30%. Rinsing diminishes the effects of ocular exposure and effects are transient and reversible (SIDS 2006).

5.2.1.1.5 Development and Reproduction

Rats given AO in diet for two generations showed no evidence of reproductive toxicity at the highest dose tested (40 mg/kg-d). There were slight increases in body weight gain in the first filial (F1) and second (F2) generations, but the dose-dependent differences were not significantly different until post-weaning. Other developmental studies in rats and rabbits have found no developmental effects below the maternally toxic dose (SIDS 2006).

5.2.1.1.6 Genotoxicity

In vitro bacterial mutagenicity studies found no evidence of mutagenicity with or without S9 metabolic activation at up to 250 µg/plate (the highest dose to not cause cytotoxicity). Clastogenic *in vivo* studies using mouse or Chinese hamster micronucleus and Chinese hamster cytogenetics assays were all negative. A dominant lethal assay in mice showed no heritable effects. An *in vitro* cell transformation assay was also negative at up to 20 µg/mL (SIDS 2006).

5.2.1.1.7 Carcinogenicity

Three carcinogenicity studies in rats or mice via various exposure routes show no evidence for carcinogenicity (SIDS 2006).

5.2.1.1.8 Neurotoxicity

No data were found.

5.2.1.1.9 Mode/Mechanism of Action

No data were found.

5.2.1.2 Ecological Data

5.2.1.2.1 Fate and Transport

AOs are nonvolatile (Ivankovic and Hrenovic 2010) and highly soluble in water (SIDS 2006).

5.2.1.2.2 Ecotoxicity

AOs demonstrate low to moderate toxicity and have low potential for bioaccumulation in terrestrial organisms. In *Phosphobacterium phosphoreum* the EC50 for luminescence after 15 minutes of exposure is 2.4 mg/L AO. In *D. magna* the EC50 is 6.8 mg/L AO (Ivankovic and Hrenovic 2010).

The range for acute aquatic EC50 values for AO are 0.60 – 32 mg/L for fish, 0.50 – 10.8 mg/L for *D. magna*, and 0.010 – 5.30 mg/L for algae. Chronic aquatic EC50 values for AO are 0.31 mg/L for fish, 0.28 mg/L for *Daphnia*, and 0.010-1.72 mg/L for algae. A chronic periphyton microcosm bioassay that included 110 algae taxa produced a no observed effect concentration (NOEC) of 0.050 mg/L AO (SIDS 2006).

No data for terrestrial species were found.

5.2.1.2.3 Degradation and Treatment

Under aerobic and anaerobic conditions, amine oxide surfactants are readily biodegradable and easily removed by conventional sewage treatment (Ivankovic and Hrenovic 2010).

5.2.2 1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,Ndimethyl-3-sulfo-, N-coco acyl derivs., hydroxides, inner salts (CAPHS C12) [CASRN 68139-30-0]

1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-coco acyl derivs., hydroxides, inner salts (CAPHS C12) [CASRN 68139-30-0], commonly known as cocoamidopropyl hydroxysultaine is a light amber soft solid (ECHA 2020c). CAPHS C12 (See Figure 4) is used in rinse-off skin cleansing products and leave-on face and neck skincare products.

Synonyms include softazoline lauramidopropyl hydroxysultaine, cocoamidopropyl hydroxysultaine, and N-(3-Cocoamidopropyl)-N,N-dimethyl-N-(2-hydroxy-3-sulfopropyl) ammonium betaine.

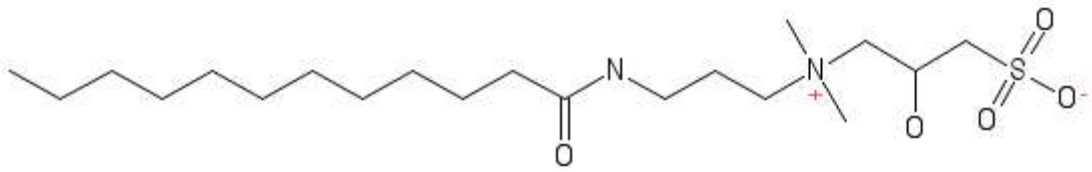

Figure 3. Structure of CAPHS C12 (PubChem Sketcher 2021)

5.2.2.1 Toxicology Data

5.2.2.1.1 Oral

In an acute oral toxicity study, Wistar rats (5/sex/group) were administered 1,000, 2,000, and 3,000 mg/kg of 42% CAPHS C12 aqueous solution (ECHA 2020c). Animals were weighed prior to dosing and on days 7 and 14 post-dosing. No deaths occurred at 1,000 mg/kg. Three of five high dose males and two of five medium dose females were found dead or euthanized due to moribundity within 24 hours of dosing. Hemorrhagic and lytic mucous membrane alternations in the GI tract, considered test-article related, were observed. Within 3 days of dosing, high dose animals exhibited reduced activity, diarrhea, abnormal postures (i.e., squatting), piloerection, and reduced skin turgor. No test-article related findings were observed macroscopically at necropsy. The LD50 for females was 2950 mg/kg at 14 days (ECHA 2020c).

The short-term effect of 36.2% CAPHS C12 in aqueous solution were evaluated in a combined repeated-dose toxicity study and reproduction/developmental toxicity screening (OECD Test NO. 422). Sprague-Dawley rats (10/sex/group) were dosed daily via oral gavage before mating, during mating, and through post-partum day 5 for females at 0, 30, 100 and 300 mg/kg-d (CIR 2017). Males were sacrificed after 5 weeks of exposure and females were sacrificed at post-partum day 6, after 6 – 8 weeks of dosing. No treatment-related mortality, body weight effects, hematological or blood chemistry changes, or organ effects (i.e., weight or macroscopically) were observed. Clinical signs of toxicity in high dose animals included hypersalivation in most animals and audible breathing three animals (either intermittent or sustained). Microscopic

changes in high dose animals included squamous cell hyperplasia, pulmonary bronchoalveolar inflammation, slight degeneration/hypertrophy of the tubular epithelium in the kidneys of males, and minimal vacuolation in some females. The NOAEL for 36.2% CAPHS C12 is 100 mg/kg-d.

5.2.2.1.2 Inhalation

No pertinent data regarding inhalation was located.

5.2.2.1.3 Dermal

In a dermal irritation study using three male New Zealand White rabbits, a 41.5% CAPHS C12 aqueous solution was applied as a single dose to a shaved 6 cm^2 area intact for 4 hours with a semi-occlusive patch (CIR 2017). Very slight erythema was observed in all animals at 1-hour post-patch removal and remained in one animal for 48 hours. CAPHS C12 was not considered a skin irritant (CIR 2017).

In a second dermal irritation study using two male and one female New Zealand White rabbits, a 16% solids aqueous solution of CAPHS C12 was applied to shaved abraded and nonabraded skin (~10% of skin surface) for 24 hours with an occlusive patch (CIR 2017). Both males showed very slight erythema at the abraded and nonabraded sites and the female showed a score 2 erythema at both abraded and nonabraded sites. Very slight edema was observed only in the female, and no reactions were observed at 72 hours. CAPHS C12 was not considered a skin irritant (CIR 2017).

In an *in vitro* skin sensitization study using the ARE-Nrf2 Luciferase Test Method, cells were exposed to 12 concentrations of CAPHS C12 for 48 hours (ECHA 2020c). CAPHS C12 produced luciferase induction >1.5 in all three iterations. The EC1.5 values were 16.48 µg/mL in the first iteration, 7.63 µg/mL in the second, and 2.5 µg/mL in the third. Based on results, CAPHS C12 is classified as a borderline nonsensitizer (ECHA 2020c).

In another skin sensitization study using the human Cell Line Activation Test (h-CLAT) method, THP-1 cells capable of expressing CD54 or CD86 were exposed to CAPHS C12 for 24 hours (ECHA 2020c). CAPHS C12 is not considered a skin sensitizer (ECHA 2020c).

In an *in vivo* skin sensitizer study, guinea pigs (20/sex/group) were dosed with CAPHS C12 (ECHA 2020c). In the first phase, animals were dosed with an intradermal injection of deionized (DI) water or 10% CAPHS C12. One week later, animals were dosed with a topical application of DI water or 100% CAPHS under occlusive conditions for 24 hours. There were no observations of skin sensitization at 24 and 48 hours after patch removal. CAPHS C12 is not a skin sensitizer (ECHA 2020c).

5.2.2.1.4 Ocular

In an ocular study using the HET-CAM assay, 4% CAPHS C12 solids in DI water (i.e., 0.3 mL CAPHS C12) was moderately irritating (CIR 2017).

In one study, three male New Zealand White rabbits were exposed to 0.1 mL of 41.5% CAPHS C12 aqueous solutions in their eyes (CIR 2017). Grade 2 to grade 3 hyperemia and edema, redness of the bulbar conjunctiva, lacrimation, and congestion and injection of the iris were all observed within the first hour and through 72 hours. Corneal and conjunctival abnormalities persisted to 14 days post-dosing and one rabbit was observed to have conjunctival chemosis at 21 days post-dosing. CAPHS C12 is a severe eye irritant (CIR 2017).

In another study, three New Zealand White rabbits were exposed to 0.1 mL of aqueous 10% solids solution CAPHS C12 (CIR 2017). Test material was administered into the right eye and not rinsed while the left eye was used as the control. Corneal opacity (score 2) was observed in all animals at 24 hours and persisted in one animal until day 7. Iridial changes were observed in one rabbit and persisted up to day 4. Conjunctival irritation was seen in two animals through day 7, with decreasing intensity and conjunctival discharge in all animals. CAPHS C12 is a severe eye irritant.

In a third study, an aqueous 16% solid solution of CAPHS C12 was administered to the right eye of three New Zealand White rabbits (CIR 2017). The treated eyes were not rinsed and the untreated left eyes were used as the control. Corneal opacity (score 2) was seen in all animals 24 hours post-dosing and persisted in one rabbit until day 7. Iridial changes were seen in two animals and persisted until day 7 in one rabbit. Conjunctival redness (score 2-3) was observed at 24 hours and persisted until day 4 in two rabbits and day 7 in one rabbit. Chemosis was seen in each animal at varying intensities. Conjunctival discharge was seen in all animals at 24 hours and at 48 hours with subsequent decreasing intensity. CAPHS C12 is a severe eye irritant (CIR 2017).

5.2.2.1.5 Development and Reproduction

In a short-term study of oral exposure to 36.2% CAPHS C12, there were no treatment-related effects on mating, fertility, or mortality (CIR 2017). Additionally, there were no relevant differences between control and treated groups in various reproductive and developmental parameters (i.e., length of gestation, number of corpora lutea, number of implantations, number of pups, live births, pre- and post-implantation loss, viability, or lactation indices). Furthermore, there were no clinical signs of treatment-related toxicity, significant effects on pup body weight, and no treatment-related findings at. The NOAEL for reproductive performance and effects on pups was 300 mg/kg-d (CIR 2017).

5.2.2.1.6 Genotoxicity

In an AMES test using up to 50% aqueous solution of CAPHS C12, the test article was not mutagenic to *S. typhimurium* TA 1535, 1537, 1538, 98, 100, and *E.coli* WP2uvrA with and without S9 activation (ECHA 2020c, CIR 2017).

Two mouse lymphoma assays using L5178Y TK+/- were run using a 36.2% aqueous solution of CAPHS C12. In the first test, CAPHS C12 was not mutagenic up to 200 µg/mL (without metabolic activation) and up to 400 µg/mL (with metabolic activation) for 3 hours. In the second test, CAPHS C12 was not mutagenic in cells exposed to up to 100 µg/mL for 24 hours (without

metabolic activation) and up to 200 µg/mL (with metabolic activation) for 3 hours. Although not mutagenic, cytotoxicity was seen in higher concentrations (CIR 2017).

Three chromosome aberration studies were run using 36.2% aqueous solution of CAPHS C12 (CIR 2017). In the first study, no chromosomal aberrations were induced when cultured human lymphocytes were exposed to up to 300 µg/mL of CAPHS C12 solution for 3 hours without metabolic activation. However, increases in the numerical aberrations were observed in the two studies where cultured human lymphocytes were exposed to up to 600 µg/mL CAPHS C12, rinsed after 3 hours of treatment, and harvested at either 20 or 44 hours after the start of exposure (CIR 2017).

5.2.2.1.7 Carcinogenicity

No pertinent data regarding carcinogenicity was located.

5.2.2.1.8 Neurotoxicity

No pertinent data regarding neurotoxicity was located.

5.2.2.1.9 Mode/Mechanism of Action

No pertinent data regarding mode or mechanism of action was located.

5.2.2.2 Ecological Data

5.2.2.2.1 Fate and Transport

The water solubility of CAPHS C12 was determined to be 520 g/L via the Loss of Drying (LOD) method (ECHA 2020c). CAPHS C12 is highly soluble at 20 degrees Celsius (˚C). The log K_{ow} for CAPHS C12 is between -0.95 and 0.53 (ECHA 2020c).

The vapor pressure of CAPHS C12 is $<3.2x10^{-3}$ mmHg at 25 ˚C indicating that it will not exist as a vapor at normal environmental temperatures (ECHA 2020c).

5.2.2.2.2 Ecotoxicity

CAPHS C12 was found to be nonhazardous and resulted in an EC50 of 11 mg/L, an NOEC of 9.2 mg/L, and a LOEC of 26 mg/L in *D. magna* (ECHA 2020c). ECOSAR modeling supports experimental data, in that LC50 values in fish, daphnia, and green algae exceed 1 mg/L.

5.2.2.2.3 Degradation and Treatment

CAPHS C12 is readily biodegradable (ECHA 2020c).

5.2.3 1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-(C8-18(even numbered) acyl) Derivs., Hydroxides, Inner Salts (CAPHC C8-18) [ECN: 939-455-3]

This substance is an alkylsultaine mixture with varying carbon chain lengths (8 – 18 even numbered carbons). A similar compound with a 12-carbon chain [CASRN 68139-30-0], cocamidopropyl hydroxysultaine (CAPHS C12; 1-Propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo,N-coco acyl derivs., hydroxides, inner salts), will be referenced as a read-across compound throughout this profile. Given that the structures of alkyl sultaines are very similar, toxicological data for CAPHS C12 can be informative about the toxicity of CAPHS C8-18 (See Figure 5). Both substances are sulfopropyl quaternary NH4 salts, which function as antistatic agents, surfactants, and skin and hair conditioning agents in cosmetics (CIR 2017).

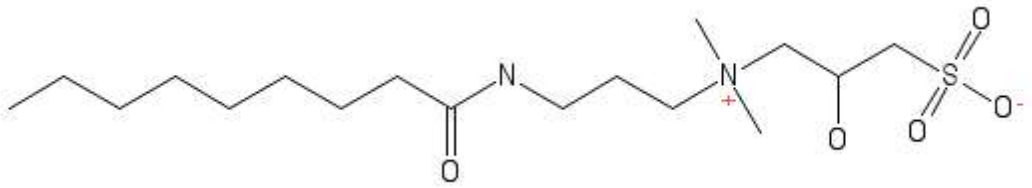

Figure 4. Structure of a 8-carbon Chain CAPHS that is Represented in the Mixture 1-propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-(C8-18(even numbered) acyl) Derivs., Hydroxides, Inner Salts (CAPHS C8-18) [ECN 939-455-3] (PubChem Sketcher 2021)

5.2.3.1 Toxicology Data

Studies used to describe the physical and chemical hazards of this substance were performed on aqueous commercial products, as it is manufactured in aqueous solutions and is not used in a solid form (ECHA 2021a).

5.2.3.1.1 Oral

No oral toxicity data specific to ECN 939-455-3 were found. In a study with 42% CAPHS C12 in aqueous solution, Wistar rats were administered 1,000, 2,000, and 3,000 mg active ingredient/kg via oral gavage and were observed for 14 days (ECHA 2021a). Up to 3 days post-dosing, general activity was reduced at 3,000 mg/kg, along with squatting position, reduced skin turgor, cyanosis, diarrhea, and piloerection on several occasions. A high incidence of pre-terminal deaths occurred at 2,000 and 3,000 mg/kg, whereas no deaths occurred at 1,000 mg/kg. At terminal sacrifice, no test-related macroscopic findings were observed in the other animals. The oral LD50 was calculated to be 2,950 mg/kg for both sexes (ECHA 2021a). This substance is Category 5 based on GHS criteria for acute oral toxicity.

Toxicity of 36.2% CAPHS C12 in aqueous solution were accessed Sprague-Dawley rats (CIR 2017). The test material was administered daily via oral gavage (0, 30, 100, or 300 mg/kg-d) before mating, during mating, and in females through postnatal day (PND) 5. In the 300 mg/kg-d dose group, microscopic changes were observed in the stomach, lungs, trachea, and kidneys.

Squamous cell hyperplasia was observed in the forestomach, most likely due to the irritant properties of the test item. The NOAEL for 36.2% CAPHS C12 was 100 mg/kg-d (CIR 2017).

5.2.3.1.2 Inhalation

No inhalation toxicity data specific to ECN 939-455-3 were found. However, the test substance is a liquid with a very low volatility, as evidenced by a low vapor pressure and a high boiling point (ECHA 2021a). Therefore exposure by the inhalation route is limited.

5.2.3.1.3 Dermal

No mortalities or clinical signs of toxicity were observed when 36.2% CAPHS C12 in solution at 2000 mg active ingredient/kg was added under semi-occlusive patches for 24-hours in Sprague-Dawley rats (CIR 2017). The dermal LD50 was >2,000 mg active ingredient/kg (CIR 2017).

CAPHS C12 was not a skin irritant in male New Zealand white rabbits when tested at concentrations up to 41.5% (ECHA 2021a, CIR 2017).

In a guinea pig maximization study, test animals were induced via intradermal injection at 10% CAPHS C12 in DI water or in Freud's adjuvant (CIR 2017). Test animals were also exposed via topical application and at challenge at 42% CAPHS C12. The substance was not sensitizing, and no skin reactions were observed (CIR 2017).

In one human study, a human repeated insulin patch test (HRIPT) was performed in 51 healthy volunteers with 0.2 mL 4% solids CAPHS C12 applied in semi-occluded patches (CIR 2017). No irritation or sensitization was observed. In another study, HRIPT was performed in 44 healthy volunteers with 0.3 mL 2.5% aqueous solution CAPHS C12 applied with an occluded patch. No skin sensitization was observed. Slight-to-moderate irritation was observed in 45% of subjects after repeat induction patches, and strong irritation reactions were observed in two subjects (CIR 2017, Consumer Product Testing Company 2017).

5.2.3.1.4 Ocular

No data on ocular toxicity and CAPHS C8-18 were located. However, one *in vitro* and multiple *in vivo* studies were performed with CAPHS C12 and are described below.

An *in vitro* hen's egg chorioallantoic membrane test (HET-CAM) assay predicted CAPHS C12 to be moderately irritating (CIR 2017).

In an eye irritation/corrosion study in male New Zealand white rabbits, 0.1 mL of CAPHS C12 as a 41.5% aqueous solution was instilled in the conjunctival sac of the right eye of each animal (ECHA 2021a, CIR 2017). Untreated eyes were used as controls. Reactions were observed up to 72 hours post treatment. Within 1 hour, grade 2 to grade 3 hyperemia and grade 2 to grade 3 edema, redness of the bulbar conjunctivae, lacrimation, and congestion and injection of the iris were observed. Some corneal and conjunctival abnormalities persisted up to 14 days post-dosing, with conjunctival chemosis observed in one rabbit up until 21 days post-dosing. It was concluded that this substance is a severe eye irritant (ECHA 2021a, CIR 2017).

In another eye irritation/corrosion study in New Zealand white rabbits, 0.1 mL CAPHS C12 as an aqueous 10% solids solution was instilled in the conjunctival sac of the right eye of each animal (ECHA 2021a, CIR 2017). After treatment, the eyes were left unrinsed and untreated eyes were the control. At 24 hours, corneal opacity (score 2) was observed in all rabbits and persisted up to day 7 in one rabbit. Iridial changes were also observed at 24 hours and persisted up to day 4 in one rabbit. Conjunctival irritation was observed through day 7 in two rabbits with decreasing intensity, and conjunctival discharge was observed in all animals. This substance was reported to be a severe eye irritant (ECHA 2021a, CIR 2017).

5.2.3.1.5 Development and Reproduction

No data regarding CAPHS C8-18 and reproductive and developmental toxicity were found.

A study described in paragraph 5.2.2.1.1 also evaluated reproductive and developmental endpoints (CIR 2017). No treatment-related effects on mating and fertility or unscheduled mortalities were observed. Additionally, there were no relevant differences between control and treatment groups in the following parameters: mean duration of gestation, mean number of corpora lutea, mean number of implantations, mean number of pups delivered, mean pre-implantation loss, and mean post-implantation loss. The NOAEL for parental (P) and filial (F) 1 generation animals was the highest tested dose: 300 mg/kg-d (CIR 2017, ECHA 2021a).

5.2.3.1.6 Genotoxicity

No data regarding CAPHS C8-18 and mutagenicity were located.

However, tests were conducted with CAPHS C12 to assess potential genotoxicity (CIR 2017). Three *in vitro* tests were conducted: an Ames test, chromosome aberration test in cultured human lymphocytes, and a mouse lymphoma assay.

In an Ames test using *S. typhimurium* TA1535, TA1537, TA1538, TA98, and TA100 strains and CAPHS C12 as a 50% aqueous solution (ECHA 2021a), two independent experiments were performed. No significant increases in the number of revertants over the respective vehicle controls were observed in any of the bacterial strains tested up to 0.2 or 0.6 µL test solution. Cytotoxic effects were observed at higher doses. Under the conditions of this assay, this substance was not mutagenic up to cytotoxic concentrations (ECHA 2021a).

In the chromosome aberrations, CAPHS C12 was negative in cultured human lymphocytes with and without metabolic activation for structural chromosome aberrations (ECHA 2021a).

In the mouse lymphoma assay, CAPHS C12 as a 36.2% aqueous solution was assessed. This substance was negative with and without metabolic activation at the highest concentrations tested of 400 µg/mL and 200 µg/mL, respectively (ECHA 2021a).

5.2.3.1.7 Carcinogenicity

No published carcinogenicity studies were found, so CAPHS C8-18 has not been classified as a carcinogen due to lack of data.

5.2.3.1.8 Neurotoxicity

No data regarding CAPHS C8-18 and neurotoxicity were found.

5.2.3.1.9 Mode/Mechanism of Action

No data regarding the mode or mechanism of action for CAPHS C8-18 were found.

5.2.3.2 Ecological Data

5.2.3.2.1 Fate and Transport

This substance has a low potential for bioaccumulation based on the low log K_{ow} of 2.1 (ECHA 2021a). No experimental studies were conducted to measure adsorption and desorption properties because the substance and its relevant degradation products decompose rapidly, and because the substance has a low log K_{ow}. Using the available log K_{ow} value, the estimated K_{oc} value is 129.42 (ECHA 2021a).

5.2.3.2.2 Ecotoxicity

Acute toxicity of this substance was investigated in two Good Laboratory Practice (GLP) - compliant studies with a freshwater species, *Brachydanio rerio*, and a seawater species, *Scophthalmus maximus*. *B. rerio* had an acute toxicity 96-hour LC50 value of 2.66 mg active content/L based on nominal concentrations (ECHA 2021a). Since a 96-hour LC50 expressed in solid content was not reported, the active content value was used as a worse-case for the assessment. S. *maximus* exhibited a 96-hour LC50 of >0.27 mg solid content/L on nominal concentrations (ECHA 2021a).

Acute toxicity to the marine crustacean species *Acartia tonsa* of the substance CAPHS C8-18 was investigated. A 48-hour EC50 value of 6.62 mg solid content/L was reported (ECHA 2021a). The toxicity to the marine alga species *Skeletonema costatum* to this substance was also investigated. The 72-hour EC50 of the substance was found to be 2.69 mg solid content/L based on nominal concentrations. The 72-hour NOEC of the substance was determined to be 0.9 mg solid content/L (ECHA 2021a).

The chronic toxicity of the test item CAPHS C8-18 (in aqueous commercial product) to the freshwater species *Pimephales promelas* was investigated in a GLP-compliant study (ECHA 2021a). The 32-day NOEC and 10% effective concentration (EC10) for hatching were 0.072 and 1.0 mg solid content/L, respectively. The 32-day NOEC and EC10 for post-hatch survival were 0.072 and 0.097 mg solid content/L, respectively. The 32-day NOEC and EC10 for larval growth (length) were 0.0082 and 0.12 mg solid content/L, respectively. The 32-day NOEC and EC10 for larval growth (weight) were <0.0082 and 0.075 mg solid content/L, respectively (ECHA 2021a).

The key value used for chemical safety assessments for freshwater fish is 0.075 mg/L (ECHA 2021a).

The chronic toxicity to the freshwater crustacean species *D. magna* to CAPHS C8-18 was investigated in a GLP compliant study (ECHA 2021a). The 21-day NOEC value was 1.39 mg solid content/L based on measured concentrations.

No data regarding terrestrial toxicity and CAPHS C8-18 was found.

5.2.3.2.3 Degradation and Treatment

This substance is rapidly degradable in the environment (ECHA 2021a).

5.2.4 Unidentified Alkylbetaine [CASRN not available]

Because no CASRN has been provided by the manufacturer, the information available for alkylbetaine is limited to what has been provided in the SDS (BIOEX 2017) and an overview of alkylbetaines used in the cosmetics industry (Burnett et al. 2018), which may or may not cover the specific constituent used in this product, but will provide some basic information on the class of compounds. This may be a fluorinated aklylbetaine, but without specific CASRN this cannot be determined. Alkylbetaines are zwitterionic compounds comprised of an inner salt generally used as a humectant or surfactant in skin, hair, or cosmetic products (Burnett et al. 2018). Figure 6 shows an example of an alkylbetaine structure.

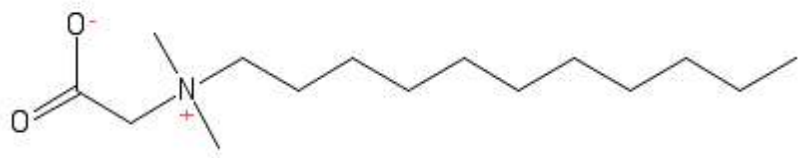

Figure 5. An Example Alkylbetaine (CASRN not available) with a Carbon Chain Length of 11 where Alkylbetaines may have Carbon Chains between 11 and 23 (Pubchem Sketcher 2021)

5.2.4.1 Toxicology Data

5.2.4.1.1 Oral

Acute oral LD50 values for several alkylbetaines ranged from 0.071 – 11.1 grams per kilogram body weight (g/kg) in rats (Burnett et al. 2018).

Chronic NOAEL values for several alkylbetaines ranged from 100 – 350 mg/kg-d and LOAEL values ranged from 150 – 500 mg/kg-d in rats (Burnett et al. 2018).

5.2.4.1.2 Inhalation

No data were found.

5.2.4.1.3 Dermal

Alkylbetaine is listed as GHS05 – corrosive with serious hazard (e.g., “danger”) (BIOEX 2017).

Dermal LD50 values were >1.3 – 16 g/kg for multiple alkylbetaines (Burnett et al. 2018). Some alkylbetaines are not sensitizing in nonhuman and human dermal studies (Burnett et al. 2018). Some alkylbetaines cause dermal irritation at 1 – 30% (Burnett et al. 2018).

5.2.4.1.4 Ocular

Alkylbetaine is listed as eye damage GHS category 1 with GHS hazard statement H318 – causes serious eye damage (BIOEX 2017).

Some alkylbetaines are ocular irritants at 10 – 30% (Burnett et al. 2018).

5.2.4.1.5 Development and Reproduction

Reproductive and developmental toxicity studies of alkylbetaines in rabbits and rats found LOAELs ranging from 10 – 300 mg/kg-d and NOAELs ranging from <10 – 300 mg/kg-d primarily due to decreased maternal body weight gain (Burnett et al. 2018).

5.2.4.1.6 Genotoxicity

Some alkylbetaines are not genotoxic via Ames, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) mutation, or chromosomal aberration assays (Burnett et al. 2018).

5.2.4.1.7 Carcinogenicity

No data were found.

5.2.4.1.8 Neurotoxicity

No data were found.

5.2.4.1.9 Mode/Mechanism of Action

Permeabilization of the stratum corneum due to solubilization of lipids may serve as a mode of action for dermal irritation (Burnett et al. 2018).

5.2.4.2 Ecological Data

5.2.4.2.1 Fate and Transport

No data were found.

5.2.4.2.2 Ecotoxicity

No data were found.

5.2.4.2.3 Degradation and Treatment

No data were found.

5.2.5 Alpha-sulfo-omega-hydroxy-poly(oxy-1,2-ethanediyl)C9-11 Alkyl Ethers, Sodium Salts (AES C9-11 1-3EO Na) [CASRN 96130-61-9]

Alpha-sulfo-omega-hydroxy-poly(oxy-1,2-ethanediyl)C9-11 alkyl ethers, sodium salts (AES C9-11 1-3EO Na) is a specific member of a carbon chain length-dependent alcohol ethoxy sulfate (AES) class of anionic surfactants. This specific CASRN is in pre-registration REACH status with ECHA, flagged as Premanufacture and Exempt from Reporting under TSCA regulations in the United States (ChemIDPlus 2009). Additionally, AES C9-11 1-3EO Na is also listed as a pesticide inert chemical for food and nonfood use by the EPA (40 CFR Part 180) (CompTox 2020d).

Synonyms include: Poly(oxy-1,2-ethanediyl), alpha-sulfo-omega-hydroxy-, C9-11-alkyl ethers, sodium salts; Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C9-11-alkyl ethers, sodium salts; including any with α or ω as ‘alpha’ or ‘a’ and ‘omega’ or ‘w.’ Alcohols, C9-11, ethoxylated, sulfates, sodium salts.

A related compound that will be referenced throughout this profile is CASRN 68891-38-3 (see paragraph 4.3.2); Poly(oxy-1,2-ethanediyl), α-sulfo-ω-hydroxy-, C12-14-alkyl ethers, sodium salts (AES C12-14 2.5EO Na). Figure 7 shows a structure of AES C9 1EO Na that is found in the mixture alpha-sulfo-omega-hydroxy-poly(oxy-1,2-ethanediyl)C9-11 alkyl ethers, sodium salts (AES C9-11 1-3EO Na; CASRN 96130-61-9).

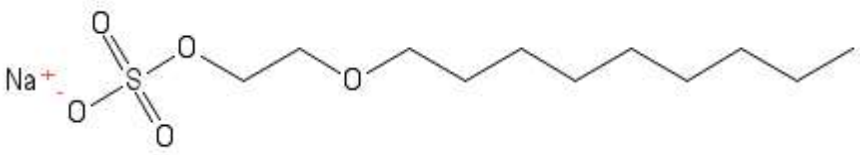

Figure 6. Structure of AES C9 1EO (PubChem Sketcher 2021)

5.2.5.1 Toxicology Data

Data specific to AES C9-11 1-3EO Na is limited and this profile will refer to data from AES class-level risk assessments and utilize read-across where appropriate.

5.2.5.1.1 Oral

No oral exposure toxicity data specific to AES C9-11 1-3EO Na were found.

Data used to represent oral acute toxicity of AES as a class is summarized in Human and Environmental Risk Assessment (HERA) (2003a) and result in LD50 estimates above 2,000 mg/kg in rats using an AES C12-14 2EO. Recoverable irritation effects and GI effects are observed for short periods following single high dose exposures.

Repeat-dose data, suggests 250 mg/kg-d as a NOAEL for rats exposed chronically and subchronically to various AES (average C12-14, EO 2.5) in drinking water, diet, and/or gavage (HERA 2003a, Little 1991). Importantly, animals exposed via gavage, similar to acute exposures, show localized stomach tissue irritation at or below this NOAEL. Systemic toxicological effects observed at higher concentrations include changes in organ weight, specifically in livers due to hepatic hypertrophy. Clinical chemistry effects were also observed at higher concentrations (HERA 2003a; Little 1991).

5.2.5.1.2 Inhalation

No inhalation toxicity data for AES C9-11 1-3EO Na were found.

Given that AES C9-11 1-3EO Na is an ion, it is unlikely to be vaporized; however, cleaning products are known to generate droplets, mists, and sprays (Clausen et al. 2020) and once dried, may be present as an inhalable powder (HERA 2003a). Read-across compounds AES C12-14 and C10-16 are identified as nonvolatile organic chemicals with unknown toxicity (Clausen et al. 2020).

In a read-across study, rats were exposed via inhalation to AES (59% solution AES C12-14 3EO NH_4) at 60 mg/L for 1 hour at 7 L/min. No mortalities occurred (HERA 2003a; Little 1991).

5.2.5.1.3 Dermal

No dermal toxicity data for NaC9-11AES were found.

According to a read-across study using AES C12-14 2.5EO Na (CAS 68891-38-3) dermal LD50 occurs at concentrations between 4,100 and 12,900 mg/kg in rabbits on both intact and abraded skin (HERA 2003a; Little 1991). In rats, dermal LD50 values were not determined as no mortality occurred in eight studies at up to 4,600 mg/kg (HERA 2003a).

Rabbits were exposed to detergents containing AES (≤27% active ingredient) for 91 days (6 hours/day, 5 days/week, 65 total exposures) and no systemic effects occurred (Petersen 1988).

A NOAEL of 12.5 mg/kg-d was determined based on applications of 0 – 2.5% solutions. Slight to moderate, transient irritation at the exposure site was noted in these treatments.

General read-across for AES indicate that dermal irritation is one of the major hazards associated with their usage (HERA 2003a; Little 1991; Robinson et al. 2010). As an example, a 4 hour exposure to 70% AES C12-14 2EO Na can show moderate-to-severe irritation initially and effects clear through 14 days and up to 21 days in different studies (HERA 2003a; Little 1991).

Dilution of AES appears to reduce dermal irritation effects (HERA 2003a; Little 1991; Robinson et al. 2010). For example, rabbits dermally exposed at 0.1 – 1% active ingredient displayed no to mild irritation, but neat application of the parent compound produced moderate-to-severe irritation.

Sensitization data specific to AES C9-11 1-3EO Na were not found. Based on read-across data with guinea pigs, AES, in general, are not expected to elicit allergic reactions (HERA 2003a).

5.2.5.1.4 Ocular

No ocular toxicity data for AES C9-11 1-3EO Na were found.

General read-across data indicate that ocular irritation is one of the major hazards associated with their usage (HERA 2003a; Little 1991; Robinson et al. 2010). As an example, AES C9-11 2.5EO Na at 32.6% applied to the eyes of rabbits produced extensive corneal damage, inflammation of the iris, and maximal conjunctival irritation that was not reversed after 7 days. Reducing concentrations to 0.1 – 1% or rinsing following exposure greatly reduced the magnitude and longevity of ocular irritation of AES exposure (HERA 2003a; Little 1991).

5.2.5.1.5 Development and Reproduction

No developmental or reproductive toxicity data for AES C9-11 1-3EO Na were found.

As a read-across, representative AES were tested in a two-generation reproduction study following OECD guidelines and GLP requirements (HERA 2003a). Data indicates that AES C9-11 1-3EO Na (27% active ingredient) is not toxic to reproduction with a NOAEL of 300 mg/kg-d (the highest treatment in the study). Slight and/or not systemic toxicological effects were observed in sperm motility, liver weights, triglyceride levels, and neutrophil counts. No embryotoxic or teratogenic effects were observed in offspring of dams exposed to 1,000 mg/kg-d (NOAEL) (HERA 2003a).

5.2.5.1.6 Genotoxicity

No genotoxicity data for AES C9-11 1-3EO Na were found.

General read-across for AES genotoxicity as a class indicates that *in vitro* and *in vivo* exposure is unlikely to produce genotoxic effects (HERA 2003a). Several AES (C12-14, C15, 2EO, 3EO Na) were tested with and without metabolic activation in *E. coli*, *S. typhimurium*, *S. cerevisiae*,

L5178Y TK+/- mouse lymphoma cells, rat liver cells, Syrian golden hamster embryo cells, and C3H 10T1/2 mouse embryo fibroblasts and all produce negative assay results (HERA 2003a).

5.2.5.1.7 Carcinogenicity

No carcinogenicity data for AES C9-11 1-3EO Na were found.

The read-across compound AES C12 3EO (cation unknown) was given to rats via drinking water for 2 years (chronic exposure) at 0.1%. There were no significant differences in tumors between the treated and control groups. Other effects, attributed to advanced age were noted in both treatments. Two potential treatment related effects were increased water intake and increased cecum:body weight in females (HERA 2003a).

5.2.5.1.8 Neurotoxicity

No neurotoxicity data for AES C9-11 1-3EO Na were found.

In a neuropharmacological study of surfactant mixtures, rats were dosed orally with a single 10 mL/kg solution of alkyl ethoxylates, alkyl ethoxy sulfates, and ethyl alcohol (Zerkle et al. 1987, Little 1991). Ataxia was observed, which triggered a follow-up study with alkyl ethoxylate alone. Similar effects in the second study suggest that AES exposure unlikely to be associated with neurotoxic effects (Little 1991).

In a battery of surfactants, zebrafish showed signs of narcotic effects at similar concentrations as toxicity for AES (C11-15 3EO, CASRN 9004-82-4) (Broening et al. 2019). Other surfactants (alkyl ethoxylates being the closest relevant comparator) produced much greater narcotic effects. In this assay, the impact on zebrafish is representative of effects observed in humans and suggest it unlikely that AES are neurological toxicants.

5.2.5.1.9 Mode/Mechanism of Action

No data on mode or mechanism of action for AES C9-11 1-3EO Na were found.

5.2.5.2 Ecological Data

5.2.5.2.1 Fate and Transport

No data on the environmental fate or transport of AES C9-11 1-3EO Na were found.

Fate and transport of AES C9-11 1-3EO Na is likely to be dominated by a high rate of biodegradation. Read-across data for slightly larger molecules (C12-14 vs. C9-11) and AES as a class suggest very complete biodegradation through multiple pathways and short half-lives in anaerobic and aerobic aquatic systems (Cowan-Ellsberry et al. 2014, Scott and Jones 2000, HERA 2003b). AES C12-14 contamination in silty-clay and clay-silty-sand soils as construction by-products indicates biodegradation in <28 days and half-lives between 6 and 9 days (Caracciolo et al. 2019).

Beyond biodegradation, the ionic nature of AES C9-11 1-3EO Na is likely the most important characteristic in predicting fate and transport. In aquatic systems, the Na cation will disassociate with the hydrophobic anionic sulfate hydrocarbon chain. The hydrophobicity is a function of the carbon chain length and ethoxylation—assuming a constant (average, based on usage) ethoxylation of 2.7. Using predicted and experimental solubility in a QSAR model produces estimates of 14,211, 4,410, and 1,369 mg/L solubility for AES C9, C10, and C11 with ethoxylation slightly increasing water solubility (Cowan-Ellsberry et al. 2014; HERA 2003b). Using a similar estimation strategy, log K_{OW} values predicted for AES C9-11 2.7EO Na are -0.56, -0.06, and 0.43. Accordingly, it is unlikely that bioaccumulation or bioconcentration of AES will be the dominant exposure pathways. Conversely, mobility of this AES in soil and aquatic environments may be high due to high solubility and low organic sorption. Larger AES (AES C12 5EO) sediment sorption data suggest K_{OC} of 1.1 ($\log_{10}(1.1)=0.04$), indicating negligible sorption with any sorption that does occur to be a function of microbial activity in the sediment (HERA 2003b, Urano et al. 1984).

As an ion, volatility and atmospheric exposure are unlikely—though aerosolized droplets of cleaning products are possible. Vapor pressures based on 2.7EO, read-across data (HERA 2003b, Cowan-Ellsberry et al. 2014), and QSAR derivation indicate 1.2e-14, 5.2e-15, and 2.2e-15 mmHg for AES C9, C10, and C11 Na, respectively. All values suggest low likelihood of atmospheric partitioning.

5.2.5.2.2 Ecotoxicity

Ecotoxicological data specific to AES C9-11 1-3EO Na were not found.

Extrapolated read-across QSAR predictions for AES with C9, C10, or C11 and 2EO lead to 48 hour acute LC50 estimates of 275, 136, and 67.4 mg/L in *Ceriodaphnia dubia* (Dyer et al. 2000). These values nearly align with the *D. magna* 48 hour acute LC50 predictions from ECOSAR (anionic surfactant special class using C9, C10, and C11 all 2.7EO estimated solubility values) (Cowan-Ellsberry et al. 2014) of 924, 91.3, and 12.9 mg/L, respectively. The QSAR from ECOSAR is likely less reliable than the Dyer et al. (2000) QSAR as it is less data rich and does not incorporate influence of the ethoxy groups, which were found to be highly significant (Dyer et al. 2000). Fish 96-hour acute LC50 predictions from ECOSAR produce the same estimates as *D. magna*. Importantly, algae appear more sensitive than fish and daphnids to AES C9, C10, and C11 Na anionic surfactants based on LC50 estimates of 3.38, 0.300, and 0.039 mg/L, respectively.

A QSAR model of chronic toxicity based on 7-day exposures in *Brachionus calyciflorus* to AES C9, C10, and C11 2EO produce LOEC estimates of 39,857, 941, and 48 mg/L (Dyer et al. 2000). NOEC estimates are 3,262, 156, and 13 mg/L, respectively. These estimates suggest lower toxicity in short-chain AES than longer chained relatives. From these QSAR estimates, (PNECs were developed, but only for AES with 12 or more carbons (HERA 2003b). Given the parabolic shape of the predictions, C9-11 would have higher PNECs and lower toxicity than their longer chained relatives (HERA 2003b).

PNECs from ECOSAR using the anionic surfactant special class model for fish (28-day exposures) and daphnids (21-day exposures) were 142, 14.0, and 1.98 mg/L for C9, C10, and

C11, respectively. Algae were significantly more sensitive: 2.41, 0.214, and 0.028 mg/L for C9, C10, and C11, respectively.

5.2.5.2.3 Degradation and Treatment

No degradation or treatment data for AES C9-11 1-3EO Na were found, but read-across from the larger class of AES demonstrates that degradation in wastewater treatment facilities occurs readily in both anaerobic and aerobic systems (HERA 2003b, Cowan-Ellsberry et al. 2014).

5.2.6 1-propanaminium, 3-amino-N-carboxymethyl) -N,N-dimethyl-,N-coco Acyl Derivs., Hydroxides, Inner Salts (CAPB) [CASRN 61789-40-0]

1-Propanaminium, 3-amino-N-carboxymethyl) -N,N-dimethyl-,N-coco acyl derivatives, hydroxides, inner salts [CASRN 61789-40-00, otherwise known as cocamidopropyl betaine (CAPB)], is a white viscous liquid (ECHA 2020b). It is an amphoteric surfactant used in personal-care products. It's predominately used as cosmetic ingredients in shampoos, cleaning agents, and hand soaps and also in household cleaning supplies such as laundry detergents, dishwashing liquids, and surface cleaners (HERA 2005). The general population may be exposed directly to CAPB from hand washing dishes and clothing, cleaning hard surfaces, or orally ingesting residue deposit on dishes. Some indirect routes of exposure are skin contact from wearing clothes, inhalation of aerosols from cleaning sprays, or drinking water (HERA 2005). Synonyms include Amphoteric L, and 3-Lauroylamidopropyl betaine. Figure 8 shows the structure of CAPB (CASRN 61789-40-0).

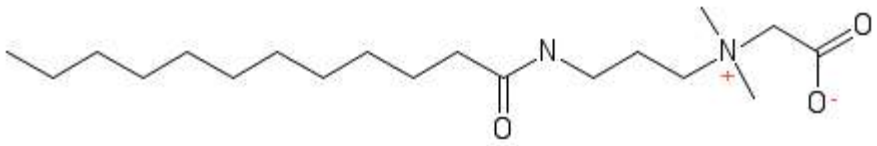

Figure 7. Structure of CAPB (PubChem Sketcher 2021)

5.2.6.1 Toxicology Data

5.2.6.1.1 Oral

Several acute oral toxicity studies in rats have been performed. In each study, CAPB was administered undiluted as a 30% active solution via oral gavage (HERA 2005). In one study, five male and five female Sprague Dawley rats were administered 5,000 mg/kg CAPB (ECHA 2020b). Animals were observed twice daily for 14 days. Body weights were recorded on days 1, 8, and 15. Piloerection and increased saliva were observed on day 1 and piloerection, hunched posture, and diarrhea on day 2. All animals recovered by day 4 and were necropsied on day 15. Transient body weight gain occurred in both sexes, but returned to normal by the second week. Gross pathology findings were normal, no mortality was observed, and the LD50 was >5,000 mg/kg (ECHA 2020b).

In another study, five male and five female Wistar rats were administered 5, 6.30, 7.94, and 10 mL/kg CAPB (Th. Goldschmidt AG 1977). On Day 1, animals receiving ≥ 5 mL/kg experienced decreased motor activity, coordination disturbance, abnormal body posture, piloerection, diarrhea, and decreased body temperature. Effects were seen 20 minutes after dosing but resolved itself after 24 hours. Redness of the stomach and intestinal mucus were observed at necropsy. The LD50 was determined to be 7,900 mg/kg (Th. Goldschmidt AG 1977).

In two other studies, five male and five female Sprague-Dawley rats were administered 5,000 mg/kg CAPB (Stepan Chemicals Co. 1982a; Stepan Chemicals Co. 1982b). Decreased motor activity, diarrhea, salivation, ataxia, and GI issues were observed early post-dosing. The LD50 is >5,000 mg/kg (Stepan Chemicals Co. 1982a; Stepan Chemicals Co. 1982b).

In another study using CD rats, five male and five female rats were administered 5,000 mg/kg CAPB (KAO Corporation 1987b). Decreased body weights, abnormal body carriage, salivation and diarrhea was observed, but all rats recovered by 4 days post-dosing. No mortality was observed. The LD50 was >5,000 mg/kg (KAO Corporation 1987b).

In another study, 4,000, 5,000, 6,300, 8,000, 16,000 mg/kg of CAPB was administered via oral exposure to five male and five female rats (Wallace 1977). Animals receiving ≥ 2,000 mg/kg experienced sluggishness, diarrhea, nasal hemorrhage, and wetness around posterior; the effects were more severe as the dose increased. The LD50 was 4,900 mg/kg (Wallace 1977).

Original sources were not found, but LD50 values of 1,800, 2,000 and 5,000 mg/kg for acute oral toxicity and 2,000 mg/kg for acute dermal toxicity was reported in EPA CompTox (CompTox 2020b). Also reported in EPA CompTox were LOAELs and NOAELs for repeated-dose oral toxicity in rats. The LOAELs are 1,000 and 300 mg/kg-d and the NOAELs are 250 and 500 mg/kg-d (CompTox 2020b).

5.2.6.1.2 Inhalation

No data regarding inhalation toxicity of CAPB were found.

5.2.6.1.3 Dermal

In an acute dermal toxicity study, five male and five female rabbits were exposed to 2,000 mg/kg of CAPB for 24 hours and observed for 14 days (KAO Corporation 1987a). The test material was applied to intact skin covering 10% of the total body surface. The only adverse observations were slightly decreased body weights in some female animals. No mortality was observed, thus the dermal LD50 is >2,000 mg/kg (KAO Corporation 1987a).

Human male volunteers (n=18) were exposed dermally via the Plastic Occlusion Stress Test (POST) technique once daily for 3 days to 7% CAPB in DI water (ECHA 2020b). On the 4th day, an occlusive plastic device was applied for 24 hours. The skin surface water loss values were 44.7 and 12.1 grams per square meter per hour ($g/m^2/h$) at 1 and 25 minutes, respectively. CAPB is considered to be a skin irritant (ECHA 2020b).

In a skin irritation test, 80% active spray dried CAPB was moistened with water and applied semiocclusively to rabbits for 4 hours and rinsed (Th. Goldschmidt AG 1991a). There were no signs of erythema or edema and CAPB was not considered an irritant (Th. Goldschmidt AG 1991a).

In another study, 30% active CAPB was applied semiocclusively to rabbits (Th. Goldschmidt AG 1990a). Application was not wiped and showed only minimal irritation after a 4-hour exposure (Th. Goldschmidt AG 1990a).

Two skin irritation tests which exposed rabbits to 30% and 25% active CAPB showed signs of moderate irritation after 4 hours (Henkel KGaA 1986a; Henkel KGaA 1987a).

In another study, 38% active CAPB was applied occlusively to rabbits for 24 hours (Goldschmidt Chemical Corporation 1993a). Application was wiped after a 24-hour exposure and CAPB was highly irritating (Goldschmidt Chemical Corporation 1993a). CAPB was shown to be mildly irritating in two studies with rabbits exposed to 10% active CAPB occlusively for 24 hours (Stepan Chemicals Co. 1982c; Stepan Chemicals Co. 1982d).

According to a summary based on exposure in animals to mixtures containing CAPB, CAPB is not a skin sensitizer (HERA 2005).

5.2.6.1.4 Ocular

In one study, New Zealand White rabbits were exposed to 0.1 mL of 50.7% CAPB into their eyes (Food and Drug Research Laboratories (FDRL) 1982). The eyes of some rabbits were rinsed after 30 seconds for 1 minute. The treated eyes were observed on days 1, 2, 3, 4, and 7. Eyes showing ocular irritation at day 7 were also observed on day 14. If still showing irritation on day 14, they were also observed on day 21. The average eye irritation index for unwashed eyes was 0.7, and 14.7 for washed eyes. CAPB is considered to be an eye irritant, and washing of the eyes helps with the reducing the irritation (FDRL 1982).

In a study using spray dried 80% active CAPB, scoring done at 24, 48 and 72 hours showed the chemical to be irreversibly irritating (Th. Goldschmidt AG 1991b). Similarly, several studies using 14 – 30% active CAPB, found CAPB to be irreversibly highly irritating (Th. Goldschmidt AG 1990b; EPA 1993; Henkel KGaA 1987b; EPA 1993).

Two additional studies using 15% and 14% active CAPB at 50% and 36% dilution were performed where CAPB without rinsing (Goldschmidt Chemical Co. 1993b; Goldschmidt Chemical Co. 1993c). Both applications were considered to be highly irritating (Goldschmidt Chemical Co. 1993b, Goldschmidt Chemical Co. 1993c).

In a study using 15% active CAPB, one eye was rinsed after 30 seconds and the other was not rinsed (EPA 1991). Rinsing had no effect on irritation, but was reversible in the rinsed eye only (EPA 1991).

Two studies assessed effects and reversibility of 10% active CAPB without rinsing. Results from suggest CPAB is a mild-to-moderate, reversible irritant (Stepan Chemicals Co. 1982e; Stepan

Chemicals Co. 1982f). Similar results were drawn from studies using 2% and 5% active CAPB (Henkel KGaA 1986c; Henkel KGaA 1986b).

5.2.6.1.5 Development and Reproduction

In a 90-day study, male and female Sprague-Dawley rats were dosed 0, 250, 500, or 1,000 mg/kg-d of CAPB via oral gavage (ECHA 2020b). No adverse reactions were observed. The reproductive toxicity NOAEL is considered to be 1,000 mg/kg-d (ECHA 2020b).

In a prenatal developmental toxicity study, female rats (25/dose) were exposed to 0, 330, 990, 3,300 mg/kg-d CAPB (28.9% active) from GD5-19 via oral gavage (CESIO 2004). No fetal incidence of skeletal variations or dose-related soft tissue variation were observed. The NOAEL for maternal toxicity is considered to be 300 mg/kg-d (HERA 2005). Since the post-implantation loss and decreased mean fetal body weight is secondary to maternal toxicity, the developmental NOAEL is 990 mg/kg-d (CESIO 2004).

5.2.6.1.6 Genotoxicity

In vitro genotoxicity tests in mammalian and bacteria cells showed no genotoxicity with 29 – 31% active CAPB (HERA 2005). Three Ames tests using up to 30% active CAPB produced negative results for *S. typhimurium* TA 98, 100, 1535, 1537 and 1538 with and without S9 activation. A mouse lymphoma test using L5178Y showed no evidence of CAPB being genotoxic (HERA 2005).

5.2.6.1.7 Carcinogenicity

CAPB has not been classified as a carcinogen due to lack of data.

5.2.6.1.8 Neurotoxicity

No data regarding neurotoxicity of CAPB were found.

5.2.6.1.9 Mode/Mechanism of Action

No data regarding the mode or mechanism of action for CAPB were found.

5.2.6.2 Ecological Data

5.2.6.2.1 Fate and Transport

Solubility data for CAPB is variable, depending on data source. Solubility ranges from >23,676 mg/L (experimental, ECHA 2020b) to 33.91 mg/L (estimated, EPA 2013). Giving precedence to experimental data over estimated values indicates that CAPB is likely to be very highly soluble in water. The log K_{OC} for CAPB is 1.531 – 2.811 (estimated, EPA 2013), indicating that it is low-to-moderately likely to adsorb to carbon-based particulates such as soil in water.

Vapor pressure is $4.81x10^{-15}$ mmHg at 25 ºC (HERA 2005), which means that CAPB would exist as a particulate in air. The K_H for CAPB is < 4x10-15 atm-m^3/mol at 25 ºC (HERA 2005), indicating that it is not volatile and will not vaporize.

CAPB has a calculated BCF value of 71 (HERA 2005; EPA 2013) indicating that it is not likely to bioconcentrate. CAPB is considered to be hydrophilic with a low log K_{OW} (-1.28) (ECHA 2020b), although estimated log K_{OW} is 2.69 and may indicate mild lipophilicity.

Fugacity modeling suggests that CAPB will exist primarily in soil (83.1%) and water (16.5%) (EPA 2013).

5.2.6.2.2 Ecotoxicity

No experimental data are available for CAPB. ECOSAR has been performed to fill the gap (EPA 2018). When CAPB is modeled as an amphoteric surfactant, it has extremely low EC/LC50 values in fish, daphnia, and algae of 0.056 mg/L (see Table 3), indicating that CAPB may be highly toxic due to its chemical properties as a zwitterionic surfactant.

No experimental or modeling data are available for plants, birds, or terrestrial toxicity outside of mammalian models listed in paragraph 5.2.6.1.1.

5.2.6.2.3 Degradation and Treatment

Biodegradation of CAPB is expected to take between 4 days and 3 weeks (EPA 2013). Removal of CAPB by wastewater treatment plants is expected to be <4% (EPA 2013).

5.2.7 Lauramine Oxide (AO C12) [CASRN 1643-20-5]

Lauramine oxide (AO C12) is a highly hygroscopic white solid. It is manufactured and supplied as an aqueous solution usually at 30 – 35% (ECHA 2020e; NCBI 2020). AO C12 is used as a foam builder, often with other amine oxides (AOs) of varying carbon chain length. The Consumer Products Information Database (CPID) lists 322 branded products (largely cleaning products) that contain up to 30% AO C12 (CPID 2020b). AO C12 is a 'green dot' surfactant on the EPA SCIL (SCIL 2020a). Reporting lists AO C12 production volume in 2015 between $1x10^7$ and $1x10^8$ pounds (Chemical Data Reporting (CDR) 2016). Synonyms include laurydimethylamine oxide (LDAO), dimethyldodecylamine-n-oxide (DDAO), dodecyldimethylamine oxide, and AO C12 (12 carbon chain amine oxide).

Toxicity data is limited for AO C12, therefore toxicity data for N,N-dimethyltetradecan-1-amine oxide (C-14 amine oxide, AO C14), a similar chemical containing two additional carbons on the long arm carbon chain, will be presented. Additionally, read-across data will be presented for structural analogues (i.e., AO C10-16, AO C12-14). Given the structural similarity of even-numbered AOs, data can be extrapolated for AO C12 with the understanding that toxicity is higher in longer-chain AO (See Figure 9) (ECHA 2020e).

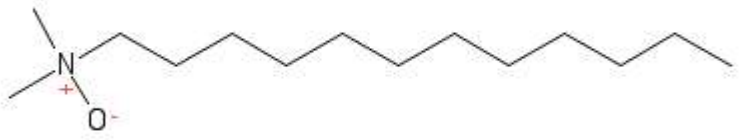

Figure 8. The Structure of Lauramine Oxide a 12-carbon Chain AO (CASRN 1643-20-5) (PubChem Sketcher 2021)

5.2.7.1 Toxicology Data

5.2.7.1.1 Oral

Male and female rats were administered single doses (420, 588, 840, 1,148, and 1,624 mg/kg) of AO C12. Animals were observed for 14 days. Gross toxic signs included decreased motor activity and salivation (all five doses groups); blanching and nasal hemorrhaging (four highest dose groups); diarrhea (four of five dose groups); and piloerection (two highest dose groups) (ECHA 2020e). Dose-dependent mortality occurred in males and females. The calculated oral LD50 was 1,064 mg/kg AO C12. As such, this chemical is classified as to OECD GHS Toxicity Category 4. The LD_{50} of 1064 mg/kg is supported by data from a separate study identifying the LD50 < 300 mg/kg (ECHA 2020e).

Male and female rats were administered AO C10-16 (0.02, 0.1, and 0.5%) in diet for 90 days. No adverse effects were observed in animals exposed to 0.02 and 0.1% (ECHA 2020d). Animals exposed to 0.5% AO C10-16 consumed less diet and experienced decreased body weight gain. As such, the NOAEL was 0.1% AO C10-16.

5.2.7.1.2 Inhalation

Male Swiss-Webster mice were exposed head-only to aerosolized droplets of 0.3% AO C12 for 10 minutes. Concentrations ranged from 0.2-5.2 mg/L. Decreased respiratory rates occurred at 1.0 and 5.2 mg/L; however, rates were even lower during post-exposure monitoring (NCBI 2020d, Review 1994). As such, decreases were not attributed to upper airway irritation. Furthermore, respiratory rate was unchanged at 0.2 mg/L. Additionally, male and female Sprague Dawley rats were exposed to aerosolized droplets of 0.3% AO C12 at 5.2 mg/L for 4 hours and observed for 14 days. No pharmatoxic signs were evident in the animals; the calculated LC50 for the aerosolized AO C12 was >5.3 mg/L.

5.2.7.1.3 Dermal

Acute dermal data are only available as read-across data. Specifically, AO C12-18 was applied as a solution in water to the skin of male and female rats. Approximately 10% of the body surface was covered for 24 hours at 2,000 mg/kg. Animals were observed immediately before exposure, shortly thereafter (at increasing intervals up to 24 hours), and for 14 days. No clinical signs or mortality were observed post-exposure (ECHA 2020e). Erythema (grade 2-4) was observed at the application site in all animals immediately after the patch was removed through test day 6; however, this was resolved by day 7. As such, the reported acute dermal LD_{50} was

>2,000 mg/kg. The LD_{50} of >2,000 mg/kg is supported by data from a separate study identifying the LD_{50} >560 mg/kg (ECHA 2020e).

Female New Zealand white rabbits were exposed to 0.4 mL AO C10-16 for 24 hours. At 24 hours, very slight to well defined erythema was observed. At 72 hours, erythema was severe (ECHA 2020e). As such, AO C10-16 is a Category 2 skin irritant.

Subchronic dermal toxicity of AO C12 was assessed in male and female mice. Repeated dermal application (5 days per week for 28 and 91 days) at 0.27 mg/application resulted in minimal to mild acanthosis (ECHA 2020e). Effects were more pronounced at the only other dose tested, 1.33 mg/application.

In another study, male and female 1 mice were exposed dermally to 5%, 10%, 15%, and 20% AO C12 for 3 weeks. Based on the severity of observations (i.e., slight-severe irritation, clinical signs of toxicity including arched spine and unkempt fur, mortality), the study was discontinued (ECHA 2020e). Instead, a separate group of mice was exposed to 0.5%, 1.0%, and 2.0% AO C12 for 4 weeks. In the second iteration of this study, animals exhibited scabs on the dorsal skin and unkempt fur, as low at 2% AO C12, thus the NOAEL was 1%.

5.2.7.1.4 Ocular

Female New Zealand white rabbits were exposed to 0.1 mL undiluted AO C10-16 (comprised of 28% AO C12) for 4 seconds. Eyes were either not rinsed or were rinsed with lukewarm distilled water. Animals were observed at 1 hour and for up to 35 days. With and without rinsing, irreversible irritation was observed. As such, AO C10-16 is a Category 1 eye irritant (ECHA 2020e).

5.2.7.1.5 Development and Reproduction

AO C12 (40, 100, and 250 mg/kg-d) was administered via oral gavage to male and female rats in a screening for reproductive and developmental toxicity. P generation males and females were dosed for at least 28 and 14 days, respectively, prior to pairing; through pairing and gestation; and until the F1 generation reached PND4. F1 generation animals were exposed embryonically and via lactation and were sacrificed on PND4. P generation males exposed to 100 and 250 mg/kg-d had reduced activity, body weight gain, and food consumption (ECHA 2020e). P generation females exposed to 250 mg/kg-d had reduced total locomotor activity and increased post-implantation loss. As such, the NOAEL for the P generation was 40 mg/kg-d. F1 generation animals exhibited effects (i.e., increased postnatal loss (PND0-4) and reduced pup weight) at doses which caused maternal toxicity (i.e., 250 mg/kg-d).

In a separate prenatal developmental toxicity study, female rats were exposed to 25 – 200 mg/kg AO C12 via oral gavage from GD9-16. Overt maternal toxicity occurred (i.e., mortality, adverse clinical signs, reduced body weight gain, and reduced feed consumption) at 100 and 200 mg/kg-d. Thus, the NOAEL for maternal toxicity was 25 mg/kg-d. Developmental toxicity was only observed at concentrations that were also maternally toxic (ECHA 2020e).

In another prenatal developmental toxicity study, female rabbits were exposed to 40 – 160 mg/kg AO C12 (96.4% of AO product mixture) via oral gavage from GD6-18. Cesarean sections were performed on GD29, and fetal weights, number of resorptions, fetal deaths and fetal morphology were evaluated. Overt maternal toxicity occurred (i.e., reduced body weight gain and feed consumption) at all three doses. No developmental effects were observed in any group. The NOAEL for maternal and developmental toxicity was >160 mg/kg (48 mg/kg-d based on active test material) (ECHA 2020e).

AO C8-16 was administered via diet to male and female rats in a 2-generation study. AO C12 made up 96.4% of the mixture. Initially, AO C8-16 was administered to P generation animals at 750, 1,500, and 3,000 ppm; however, following marked inhibition of body weight gain at the two highest levels, there doses were reduced to 375 and 188 ppm, respectively, 6 weeks into the study. Selected F1 generation animals were exposed at their parental doses through production of a F2 generation. Overall, dietary administration of AO C8-16 across two generations was associated with slight reductions in weight gain (P, F1, and F2). AO C8-16 was not associated with adverse effects on mating performance, fertility, or development of offspring (ECHA 2020e).

In a prenatal developmental toxicity study, female rats were exposed to 50 – 200 mg/kg AO C8-16 via oral gavage from GD7-17. Each dose group consisted of 32 pregnant rats; 21 were sacrificed on GD20 and fetuses were examined for morphological development. The remaining 11 rats were allowed to give birth and offspring were evaluated for viability, growth, attainment of developmental landmarks, neurobehavior and fertility. F1 generation animals were not directly dosed. Overt maternal toxicity occurred (i.e., reduced body weight gain and feed consumption) occurred at 200 mg/kg-d (ECHA 2020e). Thus, the NOAEL for maternal toxicity was 100 mg/kg-d. Fetal effects were secondary to maternal toxicity at 200 mg/kg-d; thus, the test-substance was not teratogenic, and the developmental NOAEL was 100 mg/kg-d (30 mg/kg-d based on active test material).

AO C12-18 (40, 100, and 200 mg/kg) was administered via oral gavage to P generation Sprague-Dawley rats in a reproductive and developmental screening test. Treatment with 200 mg/kg-d resulted in increased pre-implantation loss compared to control animals. Other reproductive and developmental parameters were unaffected (ECHA 2020e). As such, the NOAEL for reproductive toxicity (i.e., pre-implantation loss) was 100 mg/kg-d.

5.2.7.1.6 Genotoxicity

AO C12 was tested for mutagenicity using *S. typhimurium* (TA1535, TA1538, TA98, and TA100) in the Ames assay. With and without S9 activation, AO C12 alone was not mutagenic (Andrews et al. 1984). Similarly, AO C10-C14 was tested for mutagenicity using the Ames assay with and without S9 activation. All results were negative (ECHA 2020e).

AO C10-16 was tested in a 4- and 24-hour *in vitro* mammalian cell gene mutation test with Chinese hamster lung fibroblasts (ECHA 2020e). Experiments were conducted with and without S-9 activation for 4 and 24 hours. All results were negative.

AO C12-14 was tested in an *in vitro* micronucleus assay with human peripheral blood lymphocyte cultures. Treatments covered a range of concentrations and were performed with and without S-9 activation. AO C12-14 did not induce micronuclei when tested up to toxic concentrations with and without S-9 activation (ECHA 2020e).

In unpublished data reviewed by ECHA, AO C12 was reported negative in a rodent dominant lethal test (ECHA 2020e). Genotoxic potential was evaluated based on frequency of dead implantations, number of average live embryos, number of average implantations, and frequency of fertile mating pairs. In this test, male mice were exposed to AO C12 (10, 100, or 1,000 mg/kg-d) in water via an unspecified method (i.e., gavage, food, or water) for 5 days. Then, each male was housed with two untreated, nulliparous females for 7 days. This dosing and mating procedure repeated weekly for an additional 6 weeks to span a complete spermatogenic cycle. On GD13 or 14, pregnant females were sacrificed and total implantations, resorptions, and dead embryos were enumerated. Data suggest no dose-response or treatment-related genotoxic effects.

5.2.7.1.7 Carcinogenicity

In a study to identify tumor induction from nitrosylated amines, groups of male and female rats were administering 0.1% AO C12 with or without 0.2% sodium nitrate for 93 weeks (Lijinsky 1984). AO C12 alone did not induce an increased incidence of tumors compared to controls. There was an increased incidence of liver neoplasms in male rats concurrently administered AO C12 and nitrate. Results suggest that ingestion of AO C12 under conditions where it could undergo nitrosylation in the stomach may present an increased carcinogenic potential.

Oral and dermal carcinogenicity studies were conducted with rats and mice, respectively. Animals were continuously exposed for 2 years via feed (0.01 – 0.2%) or dermally (0.05 – 0.26%) to AO C10-16. No neoplastic or non-neoplastic treatment-related effects were identified (Cardin et al. 1985).

5.2.7.1.8 Neurotoxicity

In a screening for reproductive and developmental toxicity, male and female P generation rats were exposed to 40 – 250 mg/kg-d AO C12 via oral gavage. P generation females exposed to 250 mg/kg-d exhibited reduced total locomotor activity in a FOB (ECHA 2020e).

In a prenatal developmental toxicity study, female CD rats were exposed to 50 – 200 mg/kg AO C8-16 via oral gavage from GD7 – 17. Of 32 pregnant rats/dose, 11 rats were allowed to give birth and offspring were evaluated for viability, growth, attainment of developmental landmarks, neurobehavior, and fertility. Neurobehavior was unaffected in F1 litters in any dose group (ECHA 2020d).

5.2.7.1.9 Mode/Mechanism of Action

Similar to other surface-active chemicals, interaction of AO C14 with cell membranes and protein denaturation is the most likely source of toxic effects (Falk 2019).

Human bronchial epithelium cells were exposed to nonionic surfactants, including AO C12, with and without pharmaceutically acceptable oils. With and without oil, AO C12 was toxic at and below its critical aggregation concentrations, which were determined by surface tension measurements (Warisnoicharoen et al. 2003). Results suggest that toxicity is related to partitioning of monomeric surfactants into the cell membrane.

5.2.7.2 Ecological Data

5.2.7.2.1 Fate and Transport

AO C12 has a very high water solubility of 190,000 mg/L (NCBI 2020d) and a Level III fugacity model predicts distribution to soil (81.1%), water (15.2%), and sediment (3.66%) (EPA 2013).

AO C12 can exist in both the vapor and particulate phases (CompTox 2020c). A low rate of volatilization from surface water is expected for AO C12 based on its estimated low K_H and high water solubility (ECHA 2020e; NCBI 2020d).

AO C12 has a low estimated bioconcentration factor (CompTox 2020c). It has a low potential for bioaccumulation (ECHA 2020e) and low estimated fish biotransformation half-life of 3.36 days (CompTox 2020c).

5.2.7.2.2 Ecotoxicity

A variety of studies with fish, daphnia, and algae were conducted with AOs. These studies were described by ECHA and are summarized below. In general, algae appear to be the most sensitive group of aquatic organisms and toxieity is dependent on length of the alkyl hydrophobe (Belanger et al. 2016, EPA 2017, ECHA 2020d).

AO C12 is toxic to aquatic life. In one 96-hour semi-static test with *Danio rerio* exposed to AO C12, the 96 hour LC50 was 31.8 mg/L. Several read-across studies with fish (i.e., fathead minnows, bluegills, and rainbow trout) suggest than toxicity is higher in the longer-chain AO C14 than AO C12 (ECHA 2020e). Similarly, QSARs for cationic surfactants are related to the size of the hydrophobic component (i.e., the numbers of carbons) (EPA 2017). A full life-cycle toxicity test was conducted with fathead minnows exposed to AO C12 for 302 days under flow-through conditions. The NOEC was 0.42 mg/L based on reduced survival, egg hatch, and occluded eyes (ECHA 2020e).

Results of acute studies with the invertebrate *D. magna* support the trend seen with fish of higher toxicity with longer-chain lengths. In a 21-day survival and reproduction test with *D. magna*, the 21-day NOEC was 0.70 mg/L AO C12-14. In a 28-day freshwater periphyton microcosm assay, the NOEC was <67 ug/L (ECHA 2020e).

ErC50 (concentration at which a 50% reduction in growth rate occurs) values in studies with algae *Pseudokirchneriella subcapitata* are consistent across chain-length. In a 72-hour algal growth inhibition study with AO C12, the ErC50 was 0.266 mg/L. A similar value was reported in a 72-hour experiment with *D. subspicatus* exposed to AO C12-14 (i.e., ErC50 = 0.25 mg/L), whereas *Chlorella vulgaris* seem more resistant (i.e. ErC50 = 1.14 mg/L) (ECHA 2020e).

No studies were identified on toxicity to soil organisms (i.e., invertebrates or plants) (ECHA 2020e).

Ecologically and risk-relevant toxicity data are summarized in a paper which developed robust species sensitivity distributions (SSDs). AO C8-C16 were exposed to algae (*D. subspicatus*), an invertebrate (*D. magna*), and fish (*D. rerio*). Two additional species (macrophyte *Lemna gibba* and algae *Ankinstrodesmus flacatus*) were exposed to AO C12. The SSD 5th percentile hazardous concentration (HC5) for AO C12 was 0.052 mg/L (Belanger et al. 2016).

5.2.7.2.3 Degradation and Treatment

AO C12 does not undergo hydrolysis (ECHA 2020e).

Under aerobic and anaerobic conditions, AO C12 is rapidly degraded in the environment (i.e., aquatic environment, soil, and sediment) (ECHA 2020e).

The removal or AO C12-14 from wastewater by sewage treatment was monitored. The concentration of AO C12-14 was determined in influent and effluent samples from six municipal activated sludge treatment plants (STPs). More than 95% of AO C12-14 was removed during treatment and the level of AO C12-14 in effluent was below detection in all samples (ECHA 2020e). In a separate monitoring study of 10 STPs, percent removal of amine oxide varied widely (63 – 97%) and was inversely related to influent levels. The highest removals were observed at STPs, whereas the lowest removals were observed at oxidation ditches (ECHA 2020e).

5.2.8 Dimethyltetradecylamine Oxide (AO C14) [CASRN 3332-27-2]

N,N-dimethyltetradecan-1-amine oxide (C-14 amine oxide, AO C14) is commonly used as a surface-active component in household products and industrial processes. There are 45 branded products (largely cleaning products) that contain up to 10% AO C14 (CPID 2020a) and 16 classes of products with concentrations up to 10% (SIDS 2006). AO C14 is also a ‘green dot’ of class surfactant on the EPA SCIL. AO C14 production volume in 2015 was between $1x10^6$ and $1x10^7$ pounds (CDR 2016). AO C14 belongs to the generalized class of amphoteric surfactants, AOs. Compounds in this class are commonly used as antimicrobials (Birnie et al. 2000, Falk 2019). AO C14 is an active registry on the Toxic Substances Control Act (TSCA) list, a high production volume list (HPV List) chemical, and an exempt pesticide inert ingredient (CompTox 2020a; SRS 2020).

Synonyms for AO C14 include 1-Tetradecanamine, N,N-dimethyl-, N-oxide, myristyldimethylamine oxide, myristyldimethylamine oxide, myristamine oxide, myristyl dimethyl amine oxide, and tetradecyldimethylamine oxide (CompTox 2020a; ECHA 2020f , NCBI 2020a).

An important note about this chemical is that, as part of the larger amine oxide group, little direct toxicological data is available. The large body of interpretative and regulatory work related has relied on read-across (SIDS 2006). A similar chemical addressed in this document is the 12 carbon amine oxide: N,N-Dimethyldodecylamine-N-oxide, CAS 1643-20-5, AO C12, lauramine

oxide (NCBI 2020a; See paragraph 5.2.7). Figure 10 shows the structure of Dimethyltetradecylamine Oxide (AO C14) [CASRN 3332-27-2].

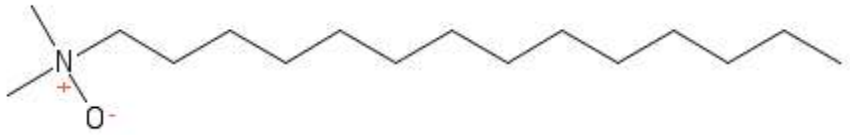

Figure 9. Structure of Dimethyltetradecylamine Oxide (AO C14) (PubChem Sketcher 2021)

5.2.8.1 Toxicology Data

5.2.8.1.1 Oral

No direct human toxicity data were found.

Oral exposure to 5,000 mg/kg (1,500 mg/kg AO C14) in male and female rats lead to no effects observed after 14 days as reported in an unpublished key study from 1997. Accordingly, an acute oral LD50 is listed as >1,500 mg/kg (ECHA 2020f).

Repeated dose studies of specifically AO C14 in mammals were not recovered, but a REACH registration dossier key study relies on a mixture of AO C10-16 as test articles for read across relevance and toxicity assessment. Importantly, in that key study, a NOAEL of 88 mg/kg-d was derived, corresponding to 0.1% active AO in diet. Endpoints impacted include reduction in food consumption (decreased palatability) and potentially cataractogenesis (2/20 in males and 2/20 in females) at the highest concentration (0.5% diet, 440 mg/kg-d). No other effects were observed after 90 days exposure in 20 female and 20 male rats at 0.02, 0.1, and 0.5% in diet (ECHA 2020f).

5.2.8.1.2 Inhalation

No data specific to AO C14 inhalation toxicity was found. See paragraph 5.2.7.1.2. for read-across inhalation studies. In sum, no pharmatoxic signs were evident in mice or rats and the calculated LC50 for the aerosolized AO C12 was >5.3 mg/L.

Data on toxic effects of AO C14 inhalation are limited by the chemical's low vapor pressure, high aqueous solubility, production and distribution in liquid solution, and resultant low likelihood of inhalation exposure (ECHA 2020f, SIDS 2006).

5.2.8.1.3 Dermal

The REACH registration dossier lists an unpublished study from 2010 that exposed rats dermally (~10% body surface) to 2,000 mg/kg of AO C12-16 mixture suspended in water. Exposure lasted 24 hours, and animals were observed for 14 days. All animals in study showed erythema up to day 7. At study end, no effects were observed externally or pathologically. Dermal LD50 estimate was derived as >2,000 mg/kg. It is unknown what amount of the AO C12-16 mixture was AO C14 (ECHA 2020f).

Additionally, 78 male and female human volunteers were exposed dermally via occlusive patch for 3 weeks to 0.75% AO (30% AO C12-14, individual isomer ratio unknown) in water. Zero sensitization reactions were observed after a 2-week rest period and subsequent challenge. Approximately 40% (31/78) showed mild erythema at the conclusion of the exposure period (ECHA 2020f).

A test of irritation in rabbits using a product with ~30% AO C14 (Stephan Company 2019) resulted in transient, but high irritation Draize scores. Additionally, animals were exposed to 0.5 mL of product for 4 hours and observed for 14 days. Maximum Draize scores (3.0 and 4.0) occurred on hours 48 and 72, but by day 7 the average score was 1.5 and by day 14, scores were 0 (ECHA 2020f).

A guinea pig sensitization test of AO C10-16 (individual isomers unknown) in water did not produce sensitization reactions. In the definitive study, 20 animals were treated to 2% product (30.4% AO C10-16) in water for 6 hours weekly for 3 weeks. In the subsequent challenge to 1% product, no positive reactions were observed (ECHA 2020f).

In summary, based on exposure in animals and humans to mixtures containing AO C14, dermal toxicity is low, irritation is dose dependent, and no sensitization reactions were observed.

5.2.8.1.4 Ocular

Rabbits were exposed to 0.1 mL of 27.8% AO C14 directly on their eye. Half the rabbits had their eyes subsequently rinsed. Rabbits were observed for 35 days. Initial Draize scores were highest in the group without a rinse, but both groups had irreversible irritation damage to corneal tissues at day 35. Iris irritation was not observed in any group, conjunctival redness was reversed in both groups by day 14, and chemosis observed in the rinse group reversed in 2 days, while in the unrinsed group was reversed in 14 days (ECHA 2020e).

5.2.8.1.5 Development and Reproduction

No data on developmental or reproductive effects specific to exposure to AO C14 were found, therefore read-across data are presented.

The key studies submitted with the REACH registration dossier of AO C14 (ECHA 2020f) were studies of AO C12-18 and AO C12-14 in rats. Both of these studies' main observations were that impacts on fetal development and reproductive success coincide with toxic effects observed in the dams and are considered nonspecific secondary toxic effects.

In the reproduction-focused study with rats, mean number of pups, litter size, and sex ratio were not impacted by treatment. Postnatal death (PND0 – 4) was observed at significantly higher rates in the 250 mg/kg-d treatment group leading to a NOAEL of 100 mg/kg-d (ECHA 2020f).

In the fetal development-focused study, female rats (25/group) were exposed GD6 – 19 to 0, 25, 100, and 200 mg/kg-d. Significant reductions in dam weight gain were observed in the 100 and 200 mg/kg-d groups, leading to derivation of the maternal toxicity NOAEL of 25 mg/kg-d. Fetal development in the 200 mg/kg-d group was reduced in number, increased in proportional rate of alterations, and delayed ossification. Delayed ossification was also observed in the 100 mg/kg-d group, leading to a NOAEL of 25 mg/kg-d for fetal toxicity (ECHA 2020f).

5.2.8.1.6 Genotoxicity

An *in vivo* micronucleus study with male and female mice exposed to a single dose of 235 mg/kg AO C14 revealed no difference in polychromatic erythrocytes' micronuclei incidence from controls after 72 hours observation (ECHA 2020f, SIDS 2006).

In vitro tests of AO C14's mutagenic capacity returned negative results in a CHO assay and Ames assays with TA100 and TA98 *S. typhimurium* strains without S9 activation (Inoue et al. 1980; SIDS 2006).

5.2.8.1.7 Carcinogenicity

No data on carcinogenic effects of AO C14 were found.

Rats and mice were exposed in diet at 0.01, 0.2. and 0.2% active ingredient and dermally to 0.05, 0.13, and 0.26% active ingredient for 104 weeks and no cancer relevant observations were noted (Cardin et al. 1985).

5.2.8.1.8 Neurotoxicity

No data were found.

5.2.8.1.9 Mode/Mechanism of Action

Similar to other surface-active chemicals, interaction of AO C14 with cell membranes and protein denaturation is the most likely source of toxic effects (Inacio et al. 2011, Falk 2019).

5.2.8.2 Ecological Data

5.2.8.2.1 Fate and Transport

AO C14 is likely to reach aqueous systems through wastewater disposal. AO C14 will biodegrade quickly in aerobic systems and slowly in anaerobic systems due to antimicrobial activity (García et al. 2007; Merrettig-Bruns and Jelen 2009).

AO C14 is predicted to have a moderately high log K_{ow} (5.66) and relatively low water solubility as a pure substance; however, amine oxide surfactants are not distributed as pure substances, but as aqueous solutions (CompTox 2020a, SIDS 2006, EPA 2020b). Accordingly, they are considered aquatically soluble and mobile in most environmental compartments as a class.

Atmospheric deposition is likely to be short due to aqueous washout and hydroxylation with < 24 hour half-life in air (CompTox 2020a, EPA 2020b).

Bioaccumulation is likely to be low, even at a moderate estimated log10 BCF value of 2.0 (CompTox 2020a, EPA 2020b) due to high rates of biodegradation and short biotransformation half-life in fish (CompTox 2020a; SCIL 2020b; SIDS 2006; García et al. 2007; Merrettig-Bruns and Jelen 2009; EPA 2020b).

5.2.8.2.2 Ecotoxicity

In a read-across review of QSAR and SSDs for amine oxide in aquatic systems paper, *D. subspicatus*, *D. magna*, and *D. rerio* individually (among other test scenarios) were exposed to AO C8-16 resulting in an HC5 for AO C12 at 0.052 mg/L. Based on the carbon chain length based QSAR, a AO C14 HC5 would approximately be one order of magnitude lower than the AO C12 value at 0.0052 mg/L (Belanger et al. 2016).

No life cycle fish tests specific to AO C14 were reported, but AO C12-14 tests in *Pimphales promelas* exposed for 302 days in flow-through conditions to 0, 0.06, 0.13, 0.25, 0.5, and 1.0 mg/L resulted in a NOEC of 0.42 mg/L observed for reduced fry survival, reduced egg hatch, and occluded eyes in test fish. In *D. magna* exposed to AO C14, at 48 hours, the acute EC50 is reported as 2.64 mg/L. A chronic (21 days) *D. magna* study using AO C12-14 at 0,0.08,0.17, 0.34, 0.70, and 1.35 mg/L (mean measured) resulted in a NOEC of 0.7 mg/L with LOEC of 1.35 mg/L and calculated EC50s of 0.96, 0.88, 1.01, and 1.04 mg/L for mortality, neonate production, brood size, and brood release timing, respectively. Algae (*P. subcapitata*) exposed to AO C14 for 72 hours resulted in EC50 on biomass of 0.095 mg/L and EC50 on growth rate of 0.19 mg/L (ECHA 2020f, SIDS 2006).

Data on terrestrial receptors was not recovered. Of note, given the fate properties of AO C14 and exposure likelihood qualification in the REACH registration dossier (ECHA 2020f, SIDS 2006), exposure in terrestrial systems is unlikely.

5.2.8.2.3 Degradation and Treatment

AO C14 will be readily biodegraded in aerobic conditions and slowly in anaerobic conditions (CompTox 2020a, SCIL 2020b, SIDS 2006, García et al. 2007, Merrettig-Bruns and Jelen 2009, EPA 2020b). Wastewater treatment predictions suggest 89% removal and 76% degradation (EPA 2020b).

5.2.9 Amines, C12-14 (Even Numbered)-alkyldimethyl, N-oxides (AO C12-14) [CASRN 308062-28-4]

AOs are surfactants consisting of a polar “head” and a hydrophobic “tail” (SIDS 2006). C12-14 are the most common alkyl chain lengths, and the average chain lengths for mixtures are 12.9 to 13.5. AOs do not exist as ‘pure’ substances, but are produced, transported, and used as aqueous solutions, typically at the 25 – 35% level of activity (SIDS 2006). AO C12-C14 are widely used as constituents of manual dishwashing detergents, shampoos, and soaps (Bonnet 2018, Schowanek 2017). They also function as foam stabilizers, thickeners and emollients, and emulsifying and conditioning agents in liquid dishwashing detergents, hard surface cleaners, fine fabric/laundry detergents, shampoos, hair conditioners, moisturizers, bar soaps, cleansing and other personal care products (SIDS 2006). Figure 11 shows the structure of a 12-carbon chain amine oxide (AO C12) likely found in the AO C12-14 mixture named amines, C12-C14-alkyldimethyl, N-oxides (CASRN 308062-28-4).

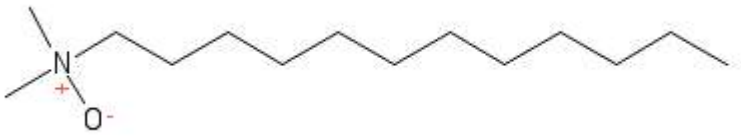

Figure 10. Structure of a 12-carbon Chain Amine Oxide (AO C12) (PubChem Sketcher 2021)

5.2.9.1 Toxicology Data

Toxicology data for AO C12-14 were limited. Chemical behaviors of AOs are expected to be very similar (Kirk-Othmer 2001). Read-across data will be presented for structural analogues (i.e., AO C10-16, AO C12-14, AO C12-18) given that these substances share similar physical chemical properties, environmental fate characteristics, ecotoxicity, and mammalian toxicity (SIDS 2006). Data can be extrapolated with the understanding that toxicity is higher in longer-chain AOs.

5.2.9.1.1 Oral

In an acute oral toxicity test with Sprague Dawley rats (5/sex/dose), animals were dosed via oral gavage once with doses of 420, 588, 840, 1148, and 1,624 mg AO C12/kg and observed for 14 days thereafter (ECHA 2020d). No deaths occurred at single doses of 600 mg AO C12/kg or less. An LD50 of 1,064 mg AO/kg was reported. Based on GHS criteria, this substance is category 4 (ECHA 2020d).

In multiple dose studies, acute oral LD50 value for rats ranged from 846 – 3,876 mg AO C13/kg. Several other AO have rat oral LD_{50}s within this range (SIDS 2006).

In a repeated dose toxicity study, rats were given doses of AO C10-16 at dosage levels of 0.02, 0.1, and 0.5% (200, 1,000, and 5,000 mg AO C10-16/kg diet) for 13 – 14 weeks (ECHA 2020d). Dietary administration of AO C10-16 produced moderate suppression of food consumption among the high dose animals, and the possibility of treatment-related cataractogenesis at the high dose level. The NOAEL was deemed to be 0.1% active AO in the diet, which equals a delivered dose of 88 mg/kg-d (ECHA 2020d).

No chronic toxicity for AO C12-14 were found.

5.2.9.1.2 Inhalation

No data regarding inhalation toxicity were located. Although inhalation exposure could occur, potential for human exposure to AOs by inhalation is minimized by its low vapor pressure and because the production, product, and industrial end use products are in aqueous solutions (SIDS 2006).

5.2.9.1.3 Dermal

There is no indication of skin sensitization for AO C12-14 based on the available animal and human data (SIDS 2006). This substance is classified as a skin irritant, and is considered to have a low hazard potential (ECHA 2020d).

Acute dermal toxicity limit tests were conducted using male and female New Zealand white rabbits (The Procter & Gamble Co. 1978). A single dose of 520 mg AO C10-16/kg (CAS 70592-80-2) were delivered to six rabbits at 27.5% purity. Three doses were applied to intact skin and three doses were applied to abraded skin. Test substance was applied to shaved areas (~25% of body surface) on backs of animals and was occluded for 24 hours. Observations were done at 24 hours and daily thereafter for 14 days. No deaths occurred at 520 mg/kg, which was equivalent to 2 mL/kg of a 30% product. Clinical results were erythema, desquamation, fissuring, eschar formation, and exfoliation of skin in all animals. Necropsy results were stomach irritation, red spots on lungs, and tan colored lungs (The Procter & Gamble Co. 1978). The number of animals used in this test was limited and the results should be taken with caution (SIDS 2006).

In another acute dermal toxicity test with male and female CD/Crl rats, animals were exposed to AO C12-18 on the back (approximately 10% of body surface) for 24 hours at 2,000 mg/kg (ECHA 2020d). Erythema (grade 2-4) was observed at the application site in all 10 animals immediately after patch removal until test day 6. A NOAEL of 2,000 mg/kg was reported based on no deaths, no oedema, no clinical abnormalities, and normal weight gain. An LD50 of AO C12-18 is > 2,000 mg AO/kg (Haferkorn 2010).

5.2.9.1.4 Ocular

No data regarding ocular toxicity and AO C12-14 were found.

Irritation from consumer products containing AO and other surfactants are moderate, transient, and reversible (SIDS 2006). However, AO C10-16, 12, 10-18, and 18 were all evaluated for eye

irritation in rabbits according to the Draize method (SIDS 2006). C12 AO was tested as produced (i.e., 30% active AO) in a rabbit eye irritation study, and there were no effects on the cornea or iris with only slight redness and swelling observed (SIDS 2006). In another study, a hair mousse product containing 0.3% AO C12 was tested for rabbit eye irritation according to the low volume modification to the Draize method, and found to be nonirritating (Pang 1994).

5.2.9.1.5 Development and Reproduction

No data were found regarding AO C12-14 and development and reproduction.

In a chronic study in which rats were given dietary doses of AO C12 in the diet over two generations, no evidence of reproductive toxicity or fertility effects were observed (SIDS 2006). The maternal and developmental NOAEL for the study was ≥37 – 128 mg AO/kg-d.

In three developmental toxicity studies where animals were exposed to two AO C14s (CAS 1643-20-5 and 70592-80-2) via oral gavage, there were no decreases in litter size, no changes in litter parameters, no malformations, and no significant differences in skeletal defects at oral doses up to 25 mg/kg-d in rats and >160 mg/kg-d in rabbits (SIDS 2006).

5.2.9.1.6 Genotoxicity

No data were found regarding AO C12-14 and genotoxicity. However, five *in vitro* Salmonella mutagenicity studies with various other AOs showed no evidence of mutagenicity either with or without S9 metabolic activation at concentrations up to 250 µg/plate (SIDS 2006). In an *in vitro* cell transformation assay, two AO (CAS 1643-20-5 and 3332-27-2) were negative at concentrations up to 20 µg/mL.

Three *in vivo* studies investigated clastogenic effects on a close structural analog of the category, 1-(methyldodecyl) dimethylamine-N-oxide (SIDS 2006). Those included a mouse micronucleus, a Chinese hamster micronucleus, and a Chinese hamster cytogenetics study. All three studies were negative, showing no increase in micronuclei or chromosome aberrations. Another *in vivo* mouse dominant lethal assay showed no evidence of heritable effects (SIDS 2006).

5.2.9.1.7 Carcinogenicity

No data regarding AO C12-14 and carcinogenicity were found. Given the structural similarity of even-numbered amine oxides, the data from the following studies below (with AO C10-16 and AO C12) can be used as a read-across.

A 2-year feeding study was conducted in rats to evaluate the carcinogenicity of AO C10-16 (The Procter & Gamble Co. 1979a). The test substance was offered in the diet to rats at concentrations of 0.01, 0.1, and 0.2% AO C10-16. Each dose group included 60 males and 60 females. There were no substance-related macroscopic changes observed and no neoplastic or non-neoplastic treatment-related effects were identified. A NOAEL of 2,000 mg AO/kg-diet was reported (The Procter & Gamble Co. 1979a).

A 2-year study in mice applied 0.1 mL of an aqueous solution of test substance three times per week at 0.05%, 0.13%, and 0.26% AO C12 to the dorsal skin of mice (The Procter & Gamble Co. 1979b). The NOAEL for dermal carcinogenicity was determined to be 3.98 mg AO C12/kg-d. The study did not result in any carcinogenic response on the exposed skin or systemically (The Procter & Gamble Co. 1979b).

5.2.9.1.8 Neurotoxicity

No data regarding neurotoxicity were found.

5.2.9.1.9 Mode/Mechanism of Action

No data regarding mode or mechanism of action were found.

5.2.9.2 Ecological Data

5.2.9.2.1 Fate and Transport

AO C12-14 are highly water soluble with an aqueous solubility of 409.5 g/L (SIDS 2006). A log Kow of <2.7 has been calculated for AO of chain length C14 and below. The measured K_{oc} of 1,525 L/kg indicates that this substance will adsorb to soil and to suspended solids and sediment in water (ECHA 2020d). AOs are not volatile and atmospheric exposure is likely to be low (SIDS 2006).

5.2.9.2.2 Ecotoxicity

Freshwater green algae are considered the most sensitive species for acute and chronic endpoints (SIDS 2006). For *Scenedesmus* and *Selenastrum*, the EC50 values range between 0.01 and 0.4 mg/L for C12 and longer chain length AO. Based on results from four reliable studies performed with AO C12-14 on *P. subcapitata (*formerly *Selenastrum)*, a 72-hour EC50 of 0.143 mg/L was calculated (ECHA 2020d). A 28-day NOEC of 0.067 mg AO/L was derived from a periphyton microcosm study in which more than 110 taxa of algae were exposed to AO C12-14 (ECHA 2020d).

Four reliable short-term toxicity studies have been conducted with AO C12-14 and fish. Each of the following studies were in static conditions for 96-hours. In a study with fathead minnows, *P. promelas*, LC50 values ranged from 2.67 to 3.46 mg AO/L depending on the source and pH of water used (ECHA 2020d). In a supporting study with Bluegill (*L. macrochirus*) an LC50 of 3.13 mg AO/L was reported (Macek and Sleight 1977). Exposure of rainbow trout (*Oncorhynchus mykiss*) resulted in a LC50 of 12.6 mg AO/L (Dommrose and Grutzmacher 1987), and exposure of zebrafish (*D. rerio*) resulted in an LC50 of 3 – 30 mg AO/L (SIDS 2006). In summary, AOs with average chain lengths ≤14 had 96-hour LC50 values in the 2 to 32 mg/L range (SIDS 2006).

In a full life-cycle toxicity test, *P. promelas* were exposed to AO C12-14 for 302 days under flow-through conditions (ECHA 2020d). The nominal test concentrations were 0, 0.06, 0.13, 0.25,

0.50, and 1.0 mg AO/L. Endpoints included survival, growth, and hatchability. The NOEC was 0.42 mg/L based on reduced fry survival, reduced egg hatch, and occluded eyes in test fish.

In a 48-hour toxicity test, *D. magna* were exposed to AO C12-14 at 0, 1.5, 3, 6, 12, and 24 mg AO/L (Noack 2001). The 48-hour EC50 was 3.1 mg/L.

A 21-day survival and reproduction test was conducted with *D. magna* exposed to AO C12-14. Mean measured test concentrations were 0, 0.08, 0.17, 0.34, 0.70, and 1.35 mg AO/L. Survival was monitored at 24 hours, 96 hours, 7 days, and daily thereafter. The 21-day NOEC and LC50 were 0.7 and 0.96 mg/L, respectively (Maki and Bishop 1979).

No data are available for terrestrial toxicity.

5.2.9.2.3 Degradation and Treatment

AO C12-14 are primarily used in household laundry and cleaning products, which are then discharged into the wastewater treatment system (SIDS 2006). Removal of amine oxide in biological wastewater treatment has been studied in laboratory simulation studies and through monitoring activities in different geographies. An OECD Test No. 303A study showed >99.8% removal. The main removal mechanism can be attributed to mineralization, and an average removal number of 98% can be assumed as applicable for secondary activated sludge treatment (SIDS 2006). Level III fugacity model predicts that release of this substance to air, soil, and water compartments results in distribution to soil (83.1%), water (16.1%), and sediment (0.8%) (ECHA 2020d).

The estimated half-life for photodegradation in air is <5.2 hours, indicating a relatively rapid atmospheric degradation potential (ECHA 2020d).

5.2.10 502W Additive (PDMS EO) [CASRN 67674-67-3]

The 2021 NRL reports were not available at the time of preparation of this document, refer to them for updated information.

502W additive contains (polyoxyethylene) propylheptamethyltrisiloxane at 70 – 90% concentration (PDMS EO) (Snow 2016). The majority ingredient in 502W additive has many synonyms including ethoxylated poly(dimethylsiloxane), ethoxylated PDMS, and 3-(polyoxyethylene)propylheptamethyltrisiloxane. Ethoxylated PDMS is a straw-colored liquid with a slight odor (Snow 2016). Likely routes of exposure to this substance are inhalation, ingestion, and skin or eye contact. This substance is a low foaming silicone surfactant, and is used in inks and coatings, agriculture, and any other applications areas where strong wetting and spreading are desired (Siltech 2019). PDMS is the dominant polymer in the silicone industry, and has been used as a fire retardant (Han et al. 2014). The ethylene oxide functional group serves to increase the aqueous solubility PDMS (Brown and Thomas 1995).

Toxicity information on PDMS EO is sparse. The manufacturer's SDS (Snow 2016) is the primary resource for this assessment and should only be used as a guide. Figure 12 shows the structure of 502W Additive Primary Ingredient PDMS EO (CASRN 67674-67-3).

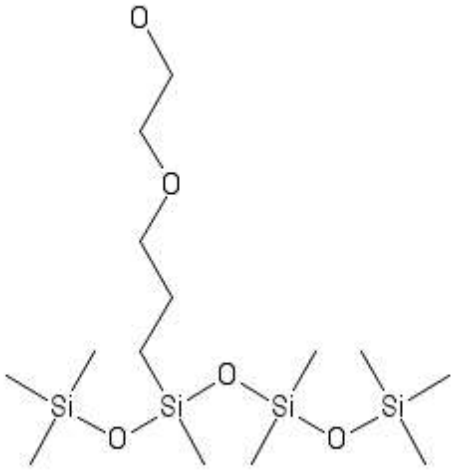

Figure 11. Structure of 502W Additive Primary Ingredient PDMS EO (CASRN 67674-67-3) (PubChem Sketcher 2021)

5.2.10.1 Toxicology Data

5.2.10.1.1 Oral

An LD50 in rats for PDMS EO was calculated to be >5,050 mg/kg with a NOAEL of 100 mg/kg (Snow 2016). According to GHS for acute toxicity categories, this substance would be a Category 5 for oral toxicity.

5.2.10.1.2 Inhalation

A 4-hour acute inhalation toxicity test was conducted in rabbits. Using a dust/mist atmosphere calculation, the LC50 was 2.3 mg/L (Snow 2016), indicating that PDMS EO is harmful if inhaled.

5.2.10.1.3 Dermal

The acute dermal toxicity of PDMS EO is estimated to be 3,049 mg/kg and an LD50 in rabbits was determined to be >2,000 – 5,000 mg/kg (Snow 2016). Based on the information available, no conclusions or classifications can be made about skin corrosion, irritation, or sensitization.

5.2.10.1.4 Ocular

There are irreversible effects on the eye in rabbits (Snow 2016).

5.2.10.1.5 Development and Reproduction

Not classified based on available information (Snow 2016).

5.2.10.1.6 Genotoxicity

An *in vitro* chromosome aberration test had negative results for genotoxicity (Snow 2016).

5.2.10.1.7 Carcinogenicity

No data were found.

5.2.10.1.8 Neurotoxicity

Not classified based on available information (Snow 2016).

5.2.10.1.9 Mode/Mechanism of Action

No data were found.

5.2.10.2 Ecological Data

5.2.10.2.1 Fate and Transport

Based on a low modeled K_H of 9.54e-5, PDMS EO is not expected to volatilize into the air compartment. The modeled half-life of PDMS EO is on the order of hours to days (<14 days). Fugacity models predict 74.3%, 13%, 12.4%, and 0.4% partition in soil, water, sediment, and air, respectively, with a very short half-life in air (10 hours), mid-range half-life in water (37.5 days), and long half-life in soil and sediment (2.5 – 11 months). Overall modeled persistence time is approximately 7 weeks (EPA 2013).

Modeled biotransformation rate constants are low, ranging from 0.001 – 0.007/day in fish and modeled BCF is high ranging from 3304 – 8459 L/kg, indicating that PDMS EO is likely lipophilic and likely to bioconcentrate. Modeled bioaccumulation factor (BAF) is in agreement with this at 2.76e6 L/kg (EPA 2013).

5.2.10.2.2 Ecotoxicity

This substance is acutely hazardous to the aquatic environment (Snow 2016). An LC50 for fish of >1 – 10 mg/L during a 96-hour exposure period and an EC50 of >1 – 10 mg/L for water fleas during a 48-hour exposure period was determined based on data from similar materials (Snow 2016). No lethal effects at saturation are expected for fish, daphnia, mysid, earthworms, and green algae based on the modeled log K_{OW} of 7.4 (EPA 2013). Modeled chronic values (geometric mean of NOEC and LOEC) based on classification as a neutral organic are 8.13e-4, 1.69e-3, and 0.02 mg/L for fish (96 hour), daphnia (48 hour), and green algae (96 hour), respectively. PDMS EO may not be soluble enough to determine chronic values for green algae. When modeled as an amphoteric surfactant, acute aquatic toxicity values are greater than 10x the aqueous solubility of PDMS EO and therefore no effect at saturation is likely.

No data for terrestrial toxicity were found.

5.2.10.2.3 Degradation and Treatment

Modeled removal at STPs is 93.96% total removal primarily via capture in primary sludge (59.84%) and waste sludge (33.34%). Minimal biodegradation (0.78%) is expected (EPA 2013).

5.2.11 Tris(2-hydroxyethyl)ammonium Dodecylsulfate (AS C12 TEA) [CASRN 139-96-8]

Triethanolamine lauryl sulfate (AS C12 TEA), also known as tris(2-hydroxyethyl)ammonium dodecylsulfate, and TEALS, is a salt comprised of two components: triethanolamine (CAS 102-71-6) and lauryl sulfate (CAS 151-41-7). AS C12 TEA is colorless liquid that is miscible with water. AS C12 TEA has a variety of uses, but is primarily used as an anionic surfactant in firefighting foams, textiles, and shampoos (CompTox 2021). Percentages of AS C12 TEA in consumer products ranges from 2.5 – 50% (CPID 2021). AS C12 TEA can be used without significant irritation below 10.5% (Fiume et al. 2013). Above 10.5%, it may cause irritation, especially if allowed to remain in contact with the skin for significant periods of time (Fiume et al. 2013).

As an ion, it is assumed complete disassociation in aquatic systems (SIDS 2007). TEA as an ion is unlikely to contribute meaningful toxicity in human and environmentally relevant systems (Könnecker et al. 2011, SIDS 2007). AS C12 TEA is a member of the larger class of anionic surfactants ASs, which are defined by linear alkyl chains (usually C8-C18), with a sulfate group head anion and a metal cation (usually Na, magnesium (Mg), NH4, or TEA, etc.) (SIDS 2007). These cations are considered low likelihood to influence toxicity and carbon chain length is to be the best predictor (SIDS 2007; Wibbertmann et al. 2011; Könnecker et al. 2011; HERA 2002a; HERA 2002b). However, the TEA salt lauryl sulfate may have reduced dermal irritation potential (Wibbertmann et al. 2011).

This section may refer to data specific to AS C12-14 TEA (C12-14 alkyl sulfate TEA, CAS 90583-18-9) as a common read-across compound. AS C12-14 TEA is addressed in paragraph 4.2.7.2.Figure 13 shows the structure of AS C12 TEA as disassociated ions in an aqueous system.

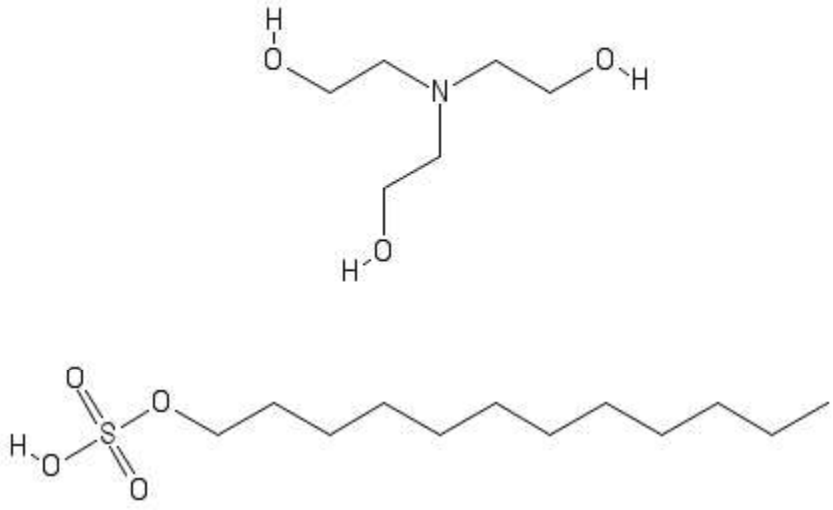

Figure 12. Structure of AS C12 TEA as Disassociated Ions in an Aqueous System (PubChem Sketcher 2021)

5.2.11.1 Toxicology Data

In general, summaries of human and environmental risk assessments of the AS class of anionic surfactants indicate low priority to human health (SIDS 2007), carbon chain dependent priority for environmental risk (SIDS 2007), and little to human or environmental systems (HERA 2002a, HERA 2002b).

5.2.11.1.1 Oral

A study in rats yielded an LD50 >2,000 mg/kg (SIDS 2007, Wibbertmann et al. 2011). This acute toxicity is similar to other >C12 AS with Na/Mg ions: those with <C12 show lower LD_{50}s overall for the cations excluding TEA (SIDS 2007, Wibbertmann et al. 2011).

No data specific to AS C12 TEA was recovered for oral repeat dose exposures. AS C12-14 TEA (CAS 90583-18-9) was used for repeat gavage exposure (28 days) in rats and led to NOAEL of 102 mg/kg-d and a LOAEL of 306 mg/kg-d (SIDS 2007; Wibbertmann et al. 2011). Effects observed include increased hemoglobin, leukocytes, and forestomach inflammation and ulceration. Effects observed in stomach were reversible in a satellite recovery group (Wibbertmann et al. 2011, SIDS 2007).

5.2.11.1.2 Inhalation

As an ion, inhalation of AS C12 TEA will rely on aerosolized droplets/mists.

Swiss albino mice exposed head only to concentrations of aerosolized solutions of AS C12 (average) TEA for 2 minutes lead to a 50% reduction in respiration rate at 135 µg/L (Little 1991;

HERA 2002a; Ciuchta and Dodd 1978). Aerosolizing solutions at 15 and 25% active ingredient leads to concentrations between 73 and 175 µg/L for AS C12 (average) TEA (and Na and NH_4) salts (Ciuchta 1976; Little 1991). These concentrations are also reported to induce between 50% and 60% respiration reduction in rabbits and mice (Little 1991; Ciuchta 1976).

5.2.11.1.3 Dermal

No data specific to AS C12 TEA and systemic toxicity after dermal exposure was recovered.

Acute dermal exposures of AS C10-16 with NH_4, Mg, Na, and K cations in rabbits (24 hours, occluded, abraded/intact, n=6-10) leads to a class-based interpretation of LD50s for AS between 200 mg/kg and > 500 mg/kg. Only AS C12 Na produced morality and corresponds with the 200 mg/kg value. All tests were performed with 25% to 33% active ingredient solutions. Moderate-to-severe irritation (erythema to necrosis lasting up to 21 days in some cases) was observed in all studies (HERA 2002a; SIDS 2007; Wibbertmann et al. 2011).

ECHA registration dossier for AS C12-14 TEA (CAS: 90583-18-9) relies on read-across from AS C8 Na and suggests dermal LD50 in rats of >2,000 mg/kg (ECHA 2021c). Approximately 10% of body area was exposed to 2,000 mg/kg AS (semi-occluded) for 24 hours. Then, skin was rinsed and animals were observed for 14 days. No effects indicating systemic toxicity were observed (ECHA 2021c). Importantly, these two read-across studies indicate increased toxicity for ≥C12 vs. C8 AS.

Mice were exposed twice-weekly via skin to AS C12-15 Na [CASRN 68890-70-0] at 0.5, 10, 12.5, and 15% active ingredient for 13 weeks. Impacts to the dermal tissue included necrosis and ulceration. Systemic impacts include modulated organ weights, decreased hemoglobin, and impacts to liver tissue structure and function (SIDS 2007; Wibbertmann et al. 2011).The NOAEL and LOAEL were 10% (~400 mg/kg) and 12.5% (~500 mg/kg), respectively (SIDS 2007; Wibbertmann et al. 2011).

The most prevalent hazard data associated with dermal AS C12 TEA exposure is irritation at high concentrations. AS C12 TEA is listed as GHS hazard class 2 (NCBI 2021). AS C12 TEA is moderately irritating to rabbit and guinea pig skin (Wibbertmann et al. 2011; SIDS 2007; Little 1991; Ciuchta and Dodd 1978). Na and NH_4 cations were tested concurrently and show severe-to-moderate irritation at similar concentrations, indicating that the TEA salts are relatively less irritating (Ciuchta and Dodd 1978). Some lines of reasoning indicate that cation does not significantly influence irritation of dermal tissue (Wibbertmann et al. 2011).

Alkyl sulfates, as a class, are generally not considered sensitizers. No data specific to AS C12 TEA was recovered, but AS C12-14 TEA (CAS: 90583-18-9) was negative for sensitization in a guinea pig maximization test (see paragraph 4.2.7.2) (Wibbertmann et al. 2011; SIDS 2007; HERAa 2002).

5.2.11.1.4 Ocular

No data on toxic effects of ocular tissues after TEALS exposure was identified.

Similar to dermal exposure, irritation is likely the most important effect of AS C12 TEA exposure to ocular tissues. AS C12 TEA has a Category 1 and Category 2 GHS scoring for serious eye damage and serious eye irritant, respectively (NCBI 2021). There is no irritation at 1.25%, slight irritation at 2%, and moderate irritation at 2.5%, 10%, and 20% applied to the eyes of rabbits in a 0.1 mL volume (Wibbertmann et al. 2011; HERA 2002a; SIDS 2007; Ciuchta and Dodd 1978; Serrano et al. 1977). Rinsing reduces irritation, and irritation was nearly reversed in 7 days (Serrano et al 1977).

Using read-across from AS C12-14 TEA, there is potential for irritation to last 14 – 21 days (ECHA 2021c).

5.2.11.1.5 Development and Reproduction

No developmental or reproductive toxicity data for AS C12 TEA was recovered.

Data for other similar AS (AS C12, C12-14, C12-15 Na) indicate maternal reproductive NOAELs of 2 to 375 mg/kg-d in rats, mice, and rabbits (Wibbertmann et al 2011; SIDS 2007; HERA 2002a). NOAELs for developmental effects range from 250 – 600 mg/kg-d. Male reproductive tissue were unaffected at 1,000 mg/kg-d (SIDS 2007). Effects on fertility were secondary to maternal bioenergetic effects or mortality. Some fetal skeletal developmental delays were observed at 600 mg/kg-d (SIDS 2007).

5.2.11.1.6 Genotoxicity

In vitro mutagenicity data indicate AS C12 TEA is negative in the Ames assay (*S. typhimurium* TA 98, TA 100) (Sunakawa et al. 1981; Wibbertmann et al. 2011).

There are no data specific to AS C12 TEA for *in vivo* mutagenicity data, but AS C12-14 TEA is negative in the OECD Test No. 474 (SIDS 2007; Wibbertmann et al. 2011; HERA 2002a).

5.2.11.1.7 Carcinogenicity

No data specific to carcinogenicity of AS C12 TEA were found.

A 2-year feeding study in rats with AS C12-15 Na is considered the definitive read-across study for alkyl sulfate and carcinogenicity (HERA 2002a; SIDS 2007; Wibbertmann et al. 2011). Wistar rats (45/sex/dose) were fed 0, 0.015, 0.15, and 1.5% active ingredient in their diet (approximately 0, 11.25, 112.5, and 1125 mg/kg-d) for 2 years. No carcinogenic effects were observed. Coarse effects observed in the highest treatment included reduced food and water intake, reduced growth rate, and a reduced rate of tumor formation. It is hypothesized that the reduced rate of tumor formation was due to the reduced food intake. Pathological findings included effects similar to other oral/dietary exposures in rats to alkyl sulfate (increased organ weights, kidney inflammation, etc.) (HERA 2002a).

5.2.11.1.8 Neurotoxicity

No data specific to neurotoxic effects of AS C12 TEA exposure were found. The read-across compound AS C12 (unknown cation) induces an increase in rat intestinal segment contractions at 1:20,000 dilution (0.005%; ~50ppm; Little 1991). No other details were available.

5.2.11.1.9 Mode/Mechanism of Action

As with other surface active compounds, and corroborated by the irritation potential of AS C12 TEA, it is likely that general effects are a function of cell membrane disruption and protein denaturation (Falk 2019).

In aquatic environments, the mode of action for toxicity is likely sorption to cell membranes and the resultant interference is the most likely source of effects at both high and low concentrations (SIDS 2007; Könnecker et al. 2011).

5.2.11.2 Ecological Data

5.2.11.2.1 Fate and Transport

Fate of AS C12 TEA in the environment is likely to be dominated by its short-half life and high rate of biodegradation of days to hours (EPA 2013, SIDS 2007, HERA 2002b, Könnecker et al. 2011, Cowan-Ellsbury et al. 2014). No data specific to AS C12 TEA was recovered, but AS C12-14 TEA (CAS 90583-18-9) is reported to complete 97 – 98% degradation in 28 days (OECD Test No. 301E) in sewage treatment plants for domestic effluent (SIDS 2007). This pattern is consistent with other alkyl sulfate (Könnecker et al. 2011, Cowan-Ellsbury et al. 2014). Soil and sediment sorption coefficients for C12 alkyl sulfates (Na cation) indicate moderate sorption with a log K_{oc} between 2.5 and 2.6 (Könnecker et al. 2011). Related, aqueous solubility decreases drastically above C12 (EPA 2013, SIDS 2007; HERA 2002b; Könnecker et al. 2011; Cowan-Ellsbury et al. 2014).

As an ion, it is unlikely that AS C12 TEA will enter atmospheric compartments and bioaccumulation is of low potential given rates of biodegradation and low bioconcentration values in fish using read-cross data from C12AS Na of 1 – 4 L/kg in *P. promelas* exposed for 4 days and 33 days (Könnecker et al. 2011).

5.2.11.2.2 Ecotoxicity

No data specific to AS C12 TEA was recovered, but the TEA cation is unlikely to produce toxicity different than other cations, and toxicity is generally best predicted by carbon chain length (SIDS 2007, HERA 2002b, Könnecker et al. 2011, Cowan-Ellsbury et al. 2014). Acute toxicity in fish is best represented by C12 LC50s of 46 and 51 mg/L in *Oryzias latipes* (two studies), 13 mg/L in *Cyprinus carpio* prelarvae, 18 mg/L in *C. carpio* eggs (96-hour EC50), and 25 mg/L in *Leuciscus idus* (96-hour EC50) (Könnecker et al. 2011). The median 48 – 96 hour LC50 across fish life stages is 25 mg/L. *D. magna* and *C. dubia* acute toxicity tests using AS C12 Na result in EC50s at 89, 25, and 5.55 mg/L for *D. magna* static, *D. magna* static, and *C. dubia* flow-through, respectively (Könnecker et al. 2011). Analytically verified long-term flow-

through AS C12 Na exposure in larval and early life-stage *P. promelas* data indicates a 42-day NOEC >1.36 mg/L (larval) and a 33-day EC_{10} of 3.6 mg/L (early life stage) (Könnecker et al. 2011). Chronic *C. dubia* exposure to AS C12 Na suggest a 7-day NOEC of 0.88 mg/L (Dyer et al. 1997, Könnecker et al. 2011). This NOEC was selected as the representative protective key value for effect estimation of AS C12 as a class (Könnecker et al. 2011). In algae (*P. subcapitata*), a 96-hour EC50 and EC_{10} of 117 and 12 mg/L for AS C12 is representative for AS C12 TEA (Cowan-Ellsbury et al. 2014). Values for more complex systems (stream mesocosms) indicate a NOEC of 0.224 mg/L for AS C12 Na (Könnecker et al. 2011). While these values are not specific to AS C12 TEA, they do provide useful context to indicate that mesocosm toxicity is likely to occur at similar concentrations as individual taxa exposures across the class of alkyl sulfates (Könnecker et al. 2011).

5.2.11.2.3 Degradation and Treatment

Given the ready aerobic and anaerobic biodegradation of alkyl sulfate as a class, it is likely that most waste water treatment will reduce AS C12 TEA concentrations (Könnecker et al. 2011; SIDS 2007; HERA 2002b). Monitoring data from WWTP can show elimination rates greater than 90% (Könnecker et al. 2011).

5.2.12 Sulfuric Acid, Mono-C12-14-alkyl Esters, Compounds with Triethanolamine (AS C12-14 TEA) [CASRN 90583-18-9]

Sulfuric acid, mono-C12-14-alkyl esters, compounds with TEA (AS C12-14 TEA), is an organosubstituted sulfate similar to Na lauryl sulfate [CASRN 102-71-6], but with the Na salt replaced with TEA. Where there are gaps in data, this assessment will rely on read-across to the 12-carbon homolog (AS C12 TEA, CASRN 139-96-8). As an ion, it is assumed to exist in complete disassociation in aquatic systems (SIDS 2007). A full assessment of the read-across homolog AS C12 TEA can be found in Section 5.2.11.

AS C12-14 TEA is made up of predictable mixtures of C12, C14, and C16 hydrophobic chain lengths: 70% C12, 25% C14, and 5% C16 (SIDS 2007). These proportions are reported under ECHA High Production Volume (HPV) Chemical Reporting Regulations for AS C12-14 TEA.

Other synonyms for AS C12-14 TEA are pluralizing acid, C12-14 ASO4 TEA, and C12, C14 AS, TEA salt. Occasionally, reference to TEA lauryl sulphate is associated with AS C12-14 TEA indicating that AS C12-14 TEA represents the “generic” or low purity product versus high purity, single carbon chain-length forms. Figure 14 shows the structure of AS C12 TEA (CASRN 139-96-8), an AS TEA with a 12-carbon chain group that is represented within the AS C12-14 TEA mixture (CASRN 90583-18-9) This compound is shown as a disassociated salt in aqueous solution.

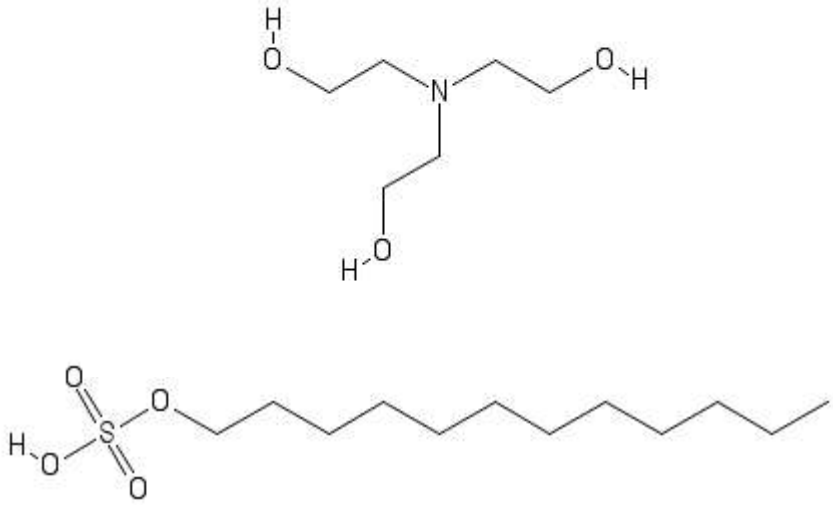

Figure 13. Structure of AS C12 TEA (CASRN 139-96-8) (PubChem Sketcher 2021)

5.2.12.1 Toxicology Data

In general, the alkyl sulfate class of anionic surfactants that AS C12-14 TEA falls under have carbon chain length-dependent priority for environmental risk (SIDS 2007) and are of no concern to human or environmental systems (HERA 2002a; HERA 2002b).

5.2.12.1.1 Oral

Acute oral exposure of male and female rats led to LD50 estimates of either >200 mg/kg or 1,000 – 2,000 mg/kg via the same primary source interpreted in two summary assessments. In this study, fasted animals were administered 20% active ingredient and observed for 10 days (Brown and Muir 1970, SIDS 2007). Diarrhea was the only reported effect. The first interpretation of this study suggests the LD50 is 1,000 – 2,000 mg/kg (Brown and Muir 1970, Little 1991, Gloxhuber and Kunstler 1992). The second interpretation reports the LD50 result as >200 mg/kg, but describe the C12-14AS (regardless of ion) as being above 2,000 mg/kg (Brown and Muir 1970, Wibbertman et al. 2011). The original data is not recoverable, but the LD50 1,000 – 2,000 mg/kg interpretation aligns with larger interpretations of the class with similar sized chains (C12-16) and cations (TEA or Na) (Wibbertman et al. 2011, SIDS 2007).

In an acute toxicity test in Wistar rats (5/sex/dose) for a read-across substance (unidentified trade name), animals were exposed to 500 or 2,000 mg/kg via gavage of aqueous solutions using a fixed dose protocol (ECHA 2021c). The LD50 was determined to be between 500 and 2,000 mg/kg. All males and one female in the 2,000 mg/kg group died in the first day of observation. No effects were observed in any animals in the 500 mg/kg group.

Acute toxicity of AS C12 TEA from unpublished data in rats resulted in an LD50 >2,000 mg/kg with no effects observed (SIDS 2007).

Repeat dose exposure of AS C12 TEA in rats has resulted in a LOAEL of 306 mg/kg-d active ingredient and a NOAEL of 102 mg/kg-d active ingredient (SIDS 2007; HERA 2002a; Wibbertmann et al 2011). In this unpublished study, rats (5/sex/dose) were exposed to 0, 70, 250, or 750 mg/kg-d of approximately 40% active ingredient aqueous solution via gavage for 28 days. At the 750 mg/kg-d (306 mg/kg-d active ingredient), effects included increased hemoglobin and leukocytes, inflammation, ulceration, acanthosis and papillomatous hyperplasia of the forestomach (severe irritation). At the NOAEL (250 mg/kg-d, 102 mg/kg-d active ingredient), GI irritation was observed but it was not considered severe and it was reversible; no other systemic effects were observed. An alternative interpretation of these data is that the irritation observed at the 250 mg/kg-d is sufficient to consider 102 mg/kg-d active ingredient a LOAEL and 28.5 mg/kg-d active ingredient a NOAEL (HERA 2002a).

Subchronic and chronic dietary exposure to the read-across substance AS C12-18 Na salts resulted in NOAELs of 55 - 252 mg/kg-d and LOAELs of 165 – 1,125 mg/kg-d (SIDS 2007).

5.2.12.1.2 Inhalation

As an ion in aqueous solution, inhalation of AS C12-14 TEA will rely on aerosolized droplets.

Swiss albino mice were exposed (head only) to 15 – 25% active ingredient concentrations of aerosolized solutions of AS C12 (average) TEA salts for 2 minutes (Ciuchta 1976; Ciuchta and Dodd 1978; Little 1991; HERA 2002a). A 50% reduction in respiration rate was reached at 135 µg/L (81-224 µg/L 95% confidence limits).

5.2.12.1.3 Dermal

No dermal exposure systemic toxicity data specific to AS C12-14 TEA were identified.

Acute dermal exposures to AS C10-16 with varying cations (none TEA) in rabbits (24 hours, occluded, abraded/intact, n=6-10) lead to a class-based interpretation of LD50s between 200 mg/kg and > 500 mg/kg using 25 – 33% active ingredient solutions. Only AS C12 Na produced mortality at 200 mg/kg. Moderate-to-severe irritation (erythema to necrosis lasting up to 21 days in some cases) was observed in all studies (HERA 2002a; SIDS 2007; Wibbertmann et al. 2011).

The dermal LD50 of AS C8 Na is > 2,000 mg/kg. Five rats received doses covering approximately 10% of body area semi-occluded for 24 hours and then, after rinsing, were observed for 14 days. No effects indicating systemic toxicity were observed (ECHA 2021c).

Mice were exposed in two sequential studies (Wibbertmann et al. 2011, ECHA 2021c; SIDS 2007; HERA 2002a) to 0.2 mL of aqueous solutions ranging from 0 –18% and 0 – 15% of AS C12-15 Na, twice weekly for 3 (0 – 18%) and 13 (0 – 15%) weeks. In the 3-week study (0 – 18%), mortality associated with dehydration was observed at 18%, leading to LOAEL and NOAEL of 15% and 10%, respectively. In the subacute study (0 – 15% for 13 weeks), a LOAEL and NOAEL of 12.5% (500 mg/kg-d) and 10% (400 mg/kg-d) were established, respectively. Systemic toxic effects observed include increased relative organ weights (liver, kidney, and heart), necrosis and ulceration of the skin, decreased hepatic glycogen and cytoplasmic

basophilia, and decreased hemoglobin. For AS C12-15 Na, limited dermal absorption is likely and systemic toxicity observed may be associated with severe irritation stress (Wibbertmann et al. 2011).

The most prevalent hazard data associated with dermal AS C12-14 TEA exposure is irritation at high concentrations. Data specific to AS C12-14 TEA indicate severely irritation (primary irritation index = 5.8 in rabbits) after 4-hour occlusive exposure (Wibbertmann et al. 2011, SIDS 2007). Similar levels of irritation were observed in rabbits and indicate irritation positively correlates with concentration (Ciuchta and Dodd 1978): concentrations of active ingredient applied in similar volumes on rabbits of 20%, 10%, and 2% lead to primary irritation indices (Draize test) of 5.2, 5, and 3.5, respectively (Ciuchta and Dodd 1978). This exposure test was repeated with Na, ammonium (NH_4), and the TEA cations in AS C12. The TEA cation is the least irritating of the cations tested—2% Na and NH_4 lead to primary irritation indices of 5-5.5 and 5.2, respectively, which correspond with indices observed at 20% TEA cation. 20% Na and NH_4 produced indices of 6 each (Ciuchta and Dodd 1978).

As a class, alkyl sulfates are not considered skin sensitizers (HERA 2002a, Wibbertmann et al. 2011, SIDS 2007; ECHA 2021c). In a guinea pig maximization test, AS C12-14 TEA is negative for skin sensitization (ECHA 2021c, SIDS 2007, HERA 2002a). In the unpublished study, 20 guinea pigs were induction treated to 5% AS C12-14 TEA by intradermal injection, then a covered patch application of 5% AS C12-14 TEA. The challenge was 1% AS C12-14 TEA and no sensitization was observed. At induction, irritation effects (redness, swelling, necrosis, etc.) were observed and the resulting challenge concentration was reduced to 1% to avoid confounding effects.

5.2.12.1.4 Ocular

No data on toxic effects of ocular tissues following AS C12-14 TEA exposure were found.

However, AS C12-14 TEA is considered highly irritating to eyes with potential for irreversible effects. Unpublished data indicate irreversible irritation effects of AS C12-14 TEA exposure in rabbit eyes, scored via Draize tests (HERA 2002a; Wimmertmann et al. 2011; SIDS 2007). Reduced concentrations of AS C12-14 TEA lead to reduced ocular irritation (Ciuchta and Dodd 1978).

Unpublished data indicates concentrations of 25% AS C12-14 TEA in rabbits leads to a classification falling between severe irritation and irritating due to a single animal showing effects (corneal opacity, score 1) lasting to 21 days. All other scored effects were reversed by day 14 observations (ECHA 2021c).

5.2.12.1.5 Development and Reproduction

No developmental or reproductive toxicology data specific to AS C12-14 TEA were found.

Data for other similar alkyl sulfates (AS C12 Na, AS C12-14 Na, AS C12-15 Na) indicate maternal reproductive NOAELs of 2 to 375 mg/kg-d in rats, mice, and rabbits via gavage during gestation (Wibbertmann et al. 2011, SIDS 2007, HERA 2002a). LOAELs for maternal animals in

the same studies range from 300 to 600 mg/kg-d. NOAELS for developmental effects range from 250 to 600 mg/kg-d. LOAELs for similar effects range from 300 to >600 mg/kg-d. Male reproductive tissue impacts were not observed, thus a NOAEL of 1,000 mg/kg-d (SIDS 2007). Effects observed on maternal fertility are largely represented by fetal losses and total litter losses accompanying maternal bioenergetic effects or mortality. Some fetal skeletal developmental delays were observed at 600 mg/kg-d (SIDS 2007).

5.2.12.1.6 Genotoxicity

AS C12-14 TEA is negative for mutagenic activity via *in vitro* and *in vivo* test systems. In the Ames reverse mutation assay, with and without metabolic activation (*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538) all data are negative (SIDS 2007, HERA 2002a, Wibbertmann et al. 2011). Cytotoxicity was observed at and above 500 µg/plate from two stages of tested concentrations (4, 20, 100, 500, and 2,500 µg/plate and 8, 40, 200, 1,000, and 5,000 µg/plate) (ECHA 2021c).

In mice exposed via oral gavage to AS C12-14 TEA, negative results were reported for the micronucleus assay (OECD Test No. 474) (SIDS 2007, HERA 2002a, Wibbertmann et al. 2011).

5.2.12.1.7 Carcinogenicity

Carcinogenicity data specific to AS C12-14 TEA were not found.

In an unpublished study summarized in several reviews, AS C12-15 Na was fed to male and female Wistar (45/sex/dose) rats at 0, 11.25, 112.5, and 1,125 mg/kg-d in diet (0, 0.015, 0.15 and 1.5%) for 2 years and no carcinogenic effects were observed (Wibbertmann et al. 2011, HERA 2002a, SIDS 2007). Coarse effects observed in the highest dose included reduced food and water intake, reduced growth rate, and reduced rate of tumor formation. It is hypothesized that this reduced rate of tumor formation was due to the reduced food intake (HERA 2002a). Pathological findings included effects similar to other oral/dietary exposures in rats to AS (i.e., increased organ weights, kidney inflammation, among others) (HERA 2002a).

5.2.12.1.8 Neurotoxicity

Neurotoxicity data specific to AS C12-14 TEA were not found.

The read-across compound AS C12 (unknown cation) induces an increase in rat intestinal segment contractions at 1:20,000 dilution (0.005%; ~50 mg/L) (Little 1991; Gale and Scott 1953).

5.2.12.1.9 Mode/Mechanism of Action

As with other surface-active compounds, and corroborated by the irritation potential of AS C12-14 TEA, it is likely that general effects are a function of cell membrane disruption and protein denaturation (Falk et al. 2019).

In aquatic environments it is likely that sorption to cell membranes and the resultant interference is the most likely source of toxic effects at both high and low concentrations (SIDS 2007; Könnecker et al. 2011).

2.12.2 Ecological Data

2.12.2.1 Fate and Transport

Environmental fate and transport of AS C12-14 TEA is best characterized by its ready biodegradability on the order of days to hours (EPA 2013, SIDS 2007, HERA 2002b). In a standardized biodegradation assay (OECD Test No. 301E), 97 – 98% was degraded in 28 days (SIDS 2007).

Outside of a short half-life in aqueous systems, AS C12-14 TEA is likely to adsorb onto sediment and soil, keeping it from reaching aqueous systems, in some cases (SIDS 2007). Longer carbon chains reduces the solubility and increase the K_{ow} coefficient. This suggests the portion of AS C12-14 TEA with C12 will have more aqueous mobility than the C14 portion. As a salt, AS C12-14 TEA is unlikely to enter atmospheric compartments (Könnecker et al. 2011). Bioaccumulation is unlikely to be a concern.

2.12.2.2 Ecotoxicity

Ecotoxicity data available for AS C12-14 TEA include LC50s for *L. idus* (freshwater Cyprinid) with a 48-hour static nominal concentration of 9.2 mg/L and a 48-hour static nominal concentration EC50 in *D. magna* of 38 mg/L (SIDS 2007). For C12 (average) AS TEA, the LC50 in *Macrones vitretus* is 1.53 mg/L (Little 1991). The overall pattern of toxicity based on chain length is parabolic: C12 is lowest, C14 is highest toxicity, and C18 toxicity approaches that of C12. For AS C12-14 TEA, it is likely that most toxicity in aquatic systems will occur due to the C14 constituent (Könnecker et al. 2011). The summary NOEC for AS C14 is 0.045 mg/L and for AS C12 is 0.88 mg/L (Könnecker et al. 2011). Values for more complex systems (i.e., stream mesocosms) indicate a NOEC of 0.106 mg/L for read-across compounds AS C12 Na and AS C14-15 Na (Könnecker et al. 2011).

2.12.2.3 Degradation and Treatment

Given the ready aerobic and anaerobic biodegradation of alkyl sulfates as a class, it is likely that most waste-water treatment will reduce AS C12-14 TEA concentrations (Könnecker et al. 2011, SIDS 2007, HERA 2002b). Monitoring data from WWTPs can show elimination rates > 90% (Könnecker et al. 2011).

5.3 Anionic Surfactants

5.3.1 Unidentified Alkyl sulfate [CASRN not available]

Alkyl sulfates (AS) are anionic surfactants predominantly used in detergents, household cleaning products, and cosmetics (Wibbertmann et al. 2011). The chemical structural features of alkyl sulfates include a linear aliphatic hydrocarbon chain, a polar sulfate group, and a counter ion.

Anionic surfactants are used to increase the efficiency of active ingredient delivery of pharmaceuticals by direct binding to the drug and/or by increasing sorption and partitioning between hydrophobic or hydrophilic organ compartments and also to remove petrochemical products from soil (Ivankovic and Hrenovic 2010). Figure 15 shows the structure of an alkyl sulfate with a 12-carbon chain. Alkyl sulfates often have hydrophilic tails with 8-18 carbons. This compound is shown as a disassociated salt

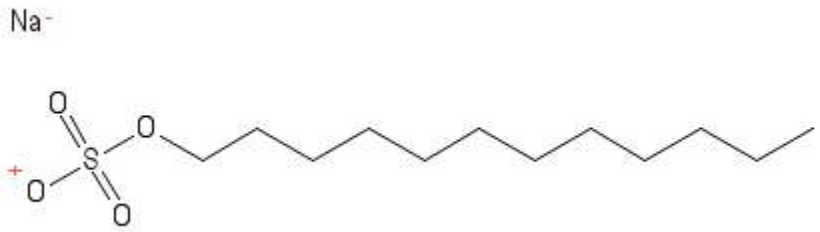

Figure 14. Structure of an Alkyl Sulfate with a 12-carbon Chain (PubChem Sketcher 2021)

5.3.1.1 Toxicology Data

5.3.1.1.1 Oral

Acute oral toxicity of alkyl sulfates is low to moderate, depending on the length of the carbon chain; shorter carbon chain lengths are more acutely toxic than longer carbon chains. LD50 values range from 290 to 580 mg/kg for C10; 1,000 to 2,000 mg/kg for C10–16; >2,000 mg/kg for C12–14, C12–15, C12–16, C12–18 and C18; and >5,000 mg/kg for C16–18 (Wibbertmann et al. 2011).

Repeated oral dosing from 3 to 104 weeks have been performed with various AS C12-18 with Na or TEA ions. Major organ effects were on the liver (e.g., increased weights, hepatomegaly, and elevated liver enzymes). For AS C16-18 Na in rats, the LOAEL for liver toxicity was determined via a 13-week dietary exposure to be 230 mg/kg-d and the NOAEL was determined from a 13-week gavage study to be 55 mg/kg-d. Commonly observed effects were reduced body weight gain, reduced food intake, reduced abdominal fat, and GI tract irritation (gavage only) (Wibbertmann et al. 2011).

5.3.1.1.2 Inhalation

No data were found.

5.3.1.1.3 Dermal

In rabbits, dermal LD50s are 200 mg/kg for AS C12 and 4,500 mg/kg for AS C12–13 and AS C10–16. Dermal exposure resulted in moderate-to-severe skin irritation and clinical symptoms including tremor, tonic–clonic convulsions, respiratory failure, and decreases in body weight (AS C12 and AS C10–16) (Wibbertmann et al. 2011).

In aqueous solutions of ~30% (AS C8-14 and AS C8-16) or 60% (AS C14-18), alkyl sulfates are corrosive to rabbit skin in dermal corrosion tests such as Organisation for Economic Co-operation and Development (OECD) Test No. 404. Medium length carbon chain AS (C12-15) are moderate to strong irritants. Longer length carbon chain AS (C16-18) were slight irritants at up to 31.5%. For context, 20% AS C12 Na is commonly used as the positive control for irritation studies on human volunteers (Wibbertmann et al. 2011).

Alkyl sulfates are not skin sensitizers (Wibbertmann et al. 2011).

5.3.1.1.4 Ocular

AS C12 at 10% are severely irritating to rabbit eyes and cause irreversible corneal effects. As the carbon chain length increases, the ocular irritating potential decreases (Wibbertmann et al. 2011).

5.3.1.1.5 Development and Reproduction

Developmental toxicity of AS C12-18 Na has been tested in rats, rabbits, and mice with effects on litter parameters not seen below maternal toxicity levels of 300 – 500 mg active ingredient/kg-d. The maternal toxicity NOAEL is 200 mg active ingredient/kg-d and the offspring NOAEL is 250 – 300 mg active ingredient/kg-d. Oral exposure to 0.1 – 1% AS C12 Na causes no adverse effects on sperm development; thus a NOAEL for male fertility of 1,000 mg active ingredient/kg-d (Wibbertmann et al. 2011).

5.3.1.1.6 Genotoxicity

In vitro and *in vivo* assays testing genotoxicity of various AS found no evidence for genotoxic potential (Wibbertmann et al. 2011).

5.3.1.1.7 Carcinogenicity

Two 2-year oral feeding studies in rats for AS C12-15 Na found no evidence of increased tumor incidence or change in spontaneous tumor type (Wibbertmann et al. 2011).

5.3.1.1.8 Neurotoxicity

No data were found.

5.3.1.1.9 Mode/Mechanism of Action

No data were found.

5.3.1.2 Ecological Data

5.3.1.2.1 Fate and Transport

No data were found.

5.3.1.2.2 Ecotoxicity

Toxicity ranges for AS in *D. magna* EC50s are 1 – 15 mg/L (Ivankovic and Hrenovic 2010).

5.3.1.2.3 Degradation and Treatment

Over 90% of alkyl sulfates are removed with secondary treatment in WWTPs, and biodegradation in water sediments is most likely due to surfactant adsorption to sediment followed by bacterial attachment and degradation (Ivankovic and Hrenovic 2010).

5.3.2 Sodium Lauryl Sulfate (AS C12 Na) [CASRN 151-21-3]

Sodium dodecyl sulfate is also known by synonyms sodium lauryl sulfate (SLS), sulfuric acid, dodecyl ester, sodium salt (1:1), and C12 Alcohol Sulfate Na (AS C12 Na). AS C12 Na is an extremely common anionic surfactant and detergent used in household and industrial products. The CPID lists 836 household brands/products containing AS C12 Na (CPID 2020b). Many of these products take advantage of its surface-active, surfactant, detergent, and wetting properties but it also has use in cosmetics, pharmaceuticals, pesticide applications (CPID 2020b, NCBI 2020a). AS C12 Na is also listed on several food additive, food contact, and GRAS U.S. regulations (21 CFR 170-186, 40 CFR 180.940) (FDA 2020, NCBI 2020a). AS C12 Na is listed as a “green circle” safer choice surfactant on the SCIL (SCIL 2020c). Industrial uses cover a wide range of manufacturing, agrochemical, biochemistry, and hydrocarbon extraction practices. Accordingly, it is an active inventory TSCA chemical, on the EPA HPV List, and present on Pesticide Chemical Search and EPA Office of Pesticide Programs Information Network Databases (SCIL 2020c).

Of note, several safety studies (dermal, inhalation) on AS C12 Na were performed by Industrial Bio-Test Laboratories, Inc., which remain unpublished. Further, this company is associated with a massive scientific misconduct scandal and should reasonably be considered suspect (Washington Post 1983). Data from these studies have been excluded from this assessment. Figure 16 shows the structure of sodium lauryl sulfate, an alcohol sulfate salt with a 12-carbon chain hydrophilic tail (AS C12 Na) [CASRN 151-21-3].

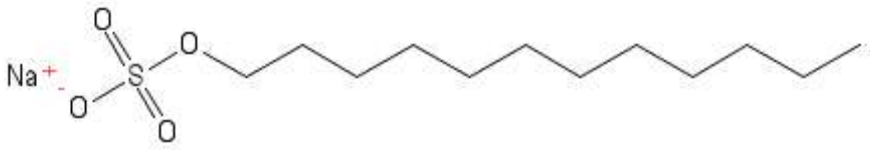

Figure 15. Structure of Sodium Lauryl Sulfate, an Alcohol Sulfate Salt with a 12-carbon Chain Hydrophilic Tail (AS C12 Na) (PubChem Sketcher 2021)

5.3.2.1 Toxicology Data

5.3.2.1.1 Oral

A reported potentially lethal oral dose for humans is 0.5 – 5 g/kg (e.g., 1 pound for a 150 pound person) from unconfirmed data (Gosselin et al. 1984).

Oral LD50 values in human-relevant animal systems are 1,200 mg/kg in rats (977 mg/kg in females and 1,427 mg/kg for males) (ECHA 2020h), 1,288 mg/kg in rats (Walker et al. 1967), 2,700 mg/kg in mice (Gloxhuber 1974), and 1,400 – 2,700 mg/kg in rats (HERA 2002a). The lowest lethality threshold in these data (1,288 mg/kg) falls into GHS Category 4.

Rats exposed to AS C12 Na 5 days per week by gavage for 28 days resulted in hepatotoxicity with a LOAEL of 300/600 mg/kg-d (dosage was increased after the second week) and a NOAEL of 100 mg/kg-d (SIDS 2007). Key effects observed include increased alanine aminotransferase (ALT) and increased liver weight. Other effects include decreased food consumption, weight gain, and hematocrit; and increased water intake, leukocytes, relative organ tissue weights, and bleeding observed in the stomach (SIDS 2007).

A 90-day study of dietary exposure in male and female rats with AS C12 Na concentrations corresponding to 0, 4, 20, 100, and 500 mg/kg-d developed a NOAEL of 100 mg/kg-d and a LOAEL of 500 mg/kg-d. The key effect observed was increased liver weight in females at the 500 mg/kg-d dose group (Walker et al. 1967; SIDS 2007).

A 21-day study of dietary exposure in rats with AS C12 Na concentrations corresponding to 0, 25, 52, 108, 208, 423, 830, and 1,643 mg/kg-d resulted in a NOAEL of 109 mg/kg-d and a LOAEL of 208 mg/kg-d. Key effects were decreased weight and body fat gain, increased relative liver weight, changes in bloodstream liver enzyme presence, and hypertrophy of the liver tissue (HERA 2002a). A similar design study extended to 90 days at 0, 59, 116, 230, 470, 950, and 1900 mg/kg-d resulted in a NOAEL of 116 mg/kg-d and LOAEL of 230 mg/kg-d. Similar effects were observed as the 28-day study (HERA 2002a).

In a chronic (2 years) exposure to AS C12 Na, no effects were observed at up to 1% of the diet in rats and a subchronic (4 months) exposure to AS C12 Na reduced growth rate at 4% of the diet (Fitzhugh and Nelson 1948). This study is summarized to a high no effect level (HNEL) of 1,000 mg/kg-d and a low effect level (LEL) of 2,000 mg/kg-d (COSMOS 2020).

The COSMOS database also references a U.S. FDA Center for Food Safety and Applied Nutrition (CFSAN) Priority-based Assessment of Food Additives (PAFA) study of AS C12 Na exposure in dogs (1 year) with a HNEL of 250 mg/kg-d and a LEL of 500 mg/kg-d (COSMOS 2020). Importantly, this original work was not recovered. As noted above, AS C12 Na is listed as GRAS and that could influence the primary availability of this study.

A short term (28 days) exposure in female mice to AS C12 Na at 2,500 mg/kg-d orally led to no significant observed effects compared to controls (Morton et al. 2004). The HNEL in mice is 2,500 mg/kg-d (COSMOS 2020).

5.3.2.1.2 Inhalation

Limited data on inhalation toxicity were found, but inhalation exposure is considered likely via airborne particulates (International Labor Organization (ILO) 2008). Aerosolized particulate exposure in guinea pigs is associated with coughing in a transient, dose-dependent manner (Zelenak et al. 1982).

Military Exposure Guidelines (MEGs) have been developed for inhalation exposure to AS C12 Na. A short-term critical MEG is 500 mg/m^3, a short-term marginal MEG is 6 mg/m^3, and a short-term negligible MEG is 1 mg/m^3 (EPA 2013).

5.3.2.1.3 Dermal

The dermal LD50 is > 2,000 mg/kg based on a study in male and female with semiocclusive coverage (ECHA 2020h).

Acute dermal (intact and abraded) exposure in male rabbits (10/group) for 24 hours lead to increased mortality in all treatments (150 to 2,000 mg/kg at 33% active ingredient) and an LD50 of 200 mg/kg active ingredient (600 mg/kg at 33% active ingredient). Effects observed included tremors, respiratory failure, and reduced weight gain (Carson and Oser 1964, SIDS 2007).

In a subchronic (90 days) study in male and female mice exposed dermally to 0, 5, 10, 12.5, and 15% active substance twice weekly, a NOAEL was established at 10% active ingredient and a LOAEL of 12.5% active ingredient. This NOAEL is describe as corresponding to 400 mg/kg-d. Effects observed were necrosis and ulceration of the skin along with changes in hematology and organ weights (ECHA 2020h).

GHS hazards include corrosive and irritant, which correspond to AS C12 Na surfactant and detergent mechanisms of action (NCBI 2020a). Damage to human skin has been well documented (Lewis 2004).

Experimental exposure of rabbit skin to 0.5 mL active ingredient led to edema and erythema observations that was not fully reversible in 72 hours (ECHA 2020h).

Unpublished manufacturer data suggests severe irritation after 4-hour occlusive exposure to 25% AS C12 Na and moderate-to-strong irritation at 5% AS C12 Na in rabbits or guinea pigs (SIDS 2007).

AS C12 Na is a common irritant control in sensitization studies (HERA 2002a).

5.3.2.1.4 Ocular

GHS hazards for irritation include eye as a target system (NCBI 2020a).

Rabbits exposed to 0.1 mL of unknown concentration AS C12 Na and observed for 21 days experienced reversible and irreversible eye irritation. Corneal opacity and conjunctivae lasted beyond 21 days (ECHA 2020h).

0.5% and 1% AS C12 Na in water was significantly irritating to rabbit eyes and injection caused severe inflammation (Grant 1986).

Ocular exposure in rabbits using the Draize method to 2, 10, and 20% AS C12 Na led to slight, moderate, and moderate irritation scores, respectively (Ciuchta and Dodd 1978).

5.3.2.1.5 Development and Reproduction

A GLP-compliant two-generation study in rats using OECD Test No. 416 at concentrations of 0, 30, 100, and 300 mg/kg-d in drinking water suggests a NOAEL > 300 mg/kg-d, as no significant treatment related effects of parents or offspring were observed. This concentration corresponds to a 0.3% level in drinking water. Observations related to test article palatability in the P generation and time to reproductive maturity in the F1 generation females were noted but not considered treatment related nor impacted overall reproductive output (ECHA 2020h).

Exposure of mothers to AS C12 Na by gavage during gestation (0, 0.2, 2, 300, or 600 mg/kg-d) to rats, mice, and rabbits (20, 20, and 13 animals/group, respectively) leads to NOAELs of 2 mg/kg-d in mothers of all taxa. LOAELs were 300 mg/kg-d for mothers due to slight mortality and other pathological observations. Of note, in rabbits, mortality was 11/13 for mothers at 600 mg/kg-d. In offspring of rats, no adverse effects were observed so a NOAEL of 600 mg/kg-d was established. In offspring of mice and rabbits, at 600 mg/kg-d (LOAEL) resorption and/or increased litter loss was observed but at 300 mg/kg-d (NOAEL) no adverse effects were observed in offspring (Palmer et al. 1975a; Palmer et al. 1975b; SIDS 2007).

Maternal LOAEL due to mortality and pathological/clinical effects at 500 mg/kg-d by gavage and NOAEL at 250 mg/kg-d. Doses in this study were 0, 63, 125, 250, and 500 mg/kg-d AS C12 Na during gestation. Teratological effects were observed in treatment groups but were not considered significant enough to establish a LOAEL (HERA 2002a; SIDS 2007).

5.3.2.1.6 Genotoxicity

All data reviewed indicate that AS C12 Na is not genotoxic in *in vitro* or *in vivo* test systems.

An *in vitro* study in *E. coli* with and without S9 fractions produced negative results at concentrations up to 2,600 µg/plate (ECHA 2020h). *In vivo* exposures in mice via gavage at doses of 0, 120, 380, or 1,200 mg/kg single doses in a rodent dominant lethal assay produced a negative genotoxic response (ECHA 2020h).

AS C12 Na is negative for mutagenicity in an Ames test (five strains of *S. typhinurium*) with and without S9 activation in studies up to 640 µg/plate (SIDS 2007).

No clastogenic effects were observed in two *in vivo* studies with 0.56 and 1.13% active ingredient AS C12 in diet for 90 days (HERA 2002a).

In vitro exposure to AS C12 Na in a mouse lymphoma cell forward mutation assay at up to 100 µg/mL (4 hours) with and without metabolic activation resulted in negative results in L5178Y TK+/- cells (McGregor et al. 1988). Of note, a cytotoxic concentration was reached at ≥70 µg/mL.

Additional negative results for AS C12 Na genotoxicity are summarized in multiple reviews (Mortelmans et al. 1986, Yam et al. 1984).

5.3.2.1.7 Carcinogenicity

Following a 704-day oral exposure to AS C12 Na at 500 mg/kg-d, no effect level was observed (Fitzhugh and Nelson 1948).

Using read-across data to support the HNEL >500 mg/kg, two studies of alkyl sulfates of varying carbon chain length (17%, AS C12 Na) with 2-year oral feeding studies in rats at 0, 0.015, 0.15, or 1.5% reveal no tumor formation. Toxic effects were observed for other noncancer endpoints (ECHA 2020b; HERA 2002a).

5.3.2.1.8 Neurotoxicity

Neurotoxicity data in human-relevant systems were not found.

Planarian behavior is impacted by AS C12 Na exposure at ~1 mg/L (Hagstrom et al. 2015).

5.3.2.1.9 Mode/Mechanism of Action

Given the surface-active/surfactant/detergent nature of the molecule, it is likely that cell membrane activity and protein denaturation is the major source of coarse toxic effects (NCBI 2020a, HERA 2002a, SIDS 2007).

A more detailed view on the dermal effects of AS C12 Na exposure mechanism indicates that rough skin is a function of reactive oxygen species (ROS) after AS C12 Na has penetrated skin cells (Mizutani et al. 2016).

5.3.2.2 Ecological Data

5.3.2.2.1 Fate and Transport

AS C12 Na has high solubility at 100,000 mg/L (Singer and Tjeerdema 1993) and log K_{ow} (1.6) and log K_{oc} (1.9) values high enough to suggest potential binding to organic compartments in aquatic and soil matrices (NCBI 2020a, EPA 2013). Fugacity model output suggests 80:20 to 66:33 soil to water distribution in systems including air, soil, water, and sediment (EPA 2013). Accordingly, mobility in groundwater should be considered slight to moderate. AS C12 Na is unlikely to volatize from wet or dry interfaces due to a low vapor pressure and ionic state (NCBI 2020a, EPA 2013). These volatilization characteristics also reduce the atmospheric presence to particulates prone to wet and dry deposition. An estimated bioconcentration factor of 71 (EPA 2013) or 6 (OPERA 2020, EPA 2013) suggests moderate to low bioconcentration, which paired with tissue biotransformation rates (EPA 2013), indicates a low likelihood of bioaccumulation.

5.3.2.2.2 Ecotoxicity

Toxicity data for ecologically relevant aquatic receptors is abundant (EPA 2020b). To facilitate interpretation, the large aquatic dataset hosted by ECOTOX has an interquartile range of 5.6 to 22.5 mg/L with a median of 12.7 mg/L. Species of note include *Daphnia* spp. and *D. rerio* near the median and *O. mykiss* near the 25th percentile. Extreme values are below 0.5 mg/L (*Dugesia japonica,* planarian) and above 1000 mg/L (*Crangon crangon*, shrimp).

Terrestrial data are limited but include EC50s of 0.0025 and 0.002% AS C12 Na in agar for nematodes based on a growth endpoint and an enzymatic half-maximal inhibition constant (IC50) value >500 mg/L in giant stockbean (*Canavalia ensiformis*) cells (EPA 2020b).

5.3.2.2.3 Degradation and Treatment

Studies designed to explore the effects of AS C12 Na on hydrocarbon degradation at environmentally relevant temperatures demonstrate that AS C12 Na will biodegrade rapidly in runoff or soil/groundwater interface (Margesin and Schinner 1998, Margesin and Schinner 1999). One specific observation of note is within approximately 21 days, 60% of AS C12 Na was mineralized by biological activity (Deschěnes 1995).

Three of five biodegradation models predict fast biodegradation, and two biodegradation models predict weeks, and days to weeks as biodegradation time windows (EPA 2013).

5.3.3 Sodium Laureth Sulfate (AES C12-14 2.5EO Na) [CASRN 68891-38-3]

A key concern associated with alcohols, C12-14, ethoxylated, sulfates, Na salts (AES C12-14 2.5EO Na, CASRN 68891-38-3) is sufficient identification. Based on the labeling in Robinson et al. (2010), AES C12-14 2.5EO Na likely represents a generic product of Na laureth sulfate (AES C12 1EO Na, CASRN 9004-82-4) and is most likely of slightly reduced purity: 12-14 carbons and an average of 2.5 ethoxy groups instead of 12 carbons and one ethoxy group. When data specific to AES C12-14 2.5EO Na are not available, data for AES C12 1EO Na will be referenced.

This compound is part of a larger class of anionic surfactants, alcohol ethoxy sulfates (alcohol ethoxysulphates, ethoxylated alcohol sulfates, alkylethoxysulfate; AES), that are used in many household cleaning products. Robinson et al. (2010) demonstrate 2,180 total consumer uses of AES C12 1EO Na at concentrations up to 50% in 2007/2008 with shampoo comprising about a third of consumer uses.

Other relevant synonyms include variations of poly(oxy-1,2-ethanediyl), α-sulfo-ω-hydroxy-, C12-14-alkyl ethers, sodium salts and/or poly(oxy-1,2-ethanediyl), α-sulfo-ω-(dodecyloxy)-, sodium salt (1:1). Figure 17 shows the structure of AES C12 1EO Na (CASRN 9004-82-4), an AES with a 12-carbon chain and one ethoxy group that is represented within the AES C12-14 1-2.5EO Na mixture (CASRN 68891-38-3).

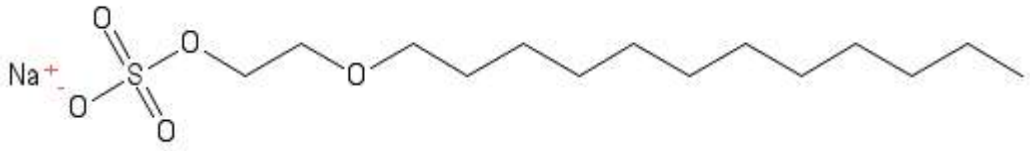

Figure 16. Structure of AES C12 1EO Na (PubChem Sketcher 2021)

5.3.3.1 Toxicology Data

AES, as a class, are considered of no concern to both human and environmental systems (HERA 2003a; HERA 2003b) either by low exposure or low toxicity. AES are largely assessed as a class. AES C12-14 2.5EO Na is on the EPA SCIL as a 'green circle' surfactant, indicating low toxicity and/or high biodegradation rates (SCIL 2020c).

5.3.3.1.1 Oral

Reported oral, acute LD50s are >2,000 and >2,500 mg/kg (data summarized in HERA 2003a; largely sourced from Little 1991) and 2,870 mg/kg in rats (ECHA 2020g). The key study referenced in ECHA registration dossier followed OECD Test No. 401 and observed mortalities in rats exposed to 4,000 and 5,000 mg/kg active ingredient and decreased respiration, pallor of extremities, and increased salivation at 3,200 mg active ingredient/kg. Effects observed at 2,000 mg/kg (piloerection, hunched posture, lethargy, and diarrhea) were attributed to reversible irritation (ECHA 2020g). A second study corroborates the irritation hypothesis by observing rats for longer periods of time after a 2,000 mg/kg dose. Effects were observed for only 4 hours (ECHA 2020g) and rats recovered.

Walker et al. (1967) exposed rats to naturally and synthetically derived, AES C12-15 3EO (salt unknown) in both acute and repeat exposures. LD50s were estimated for the naturally and synthetically derived AES at 1,995 and 2,138 mg/kg, respectively.

In a 90-day repeat dose study, male and female rats (ECHA 2020g) were exposed to 25, 75, and 225 mg/kg-d active ingredient (70% of product). The concentrations used in this study were derived from a prior subchronic (28 days) exposure of rats to 100, 500, and 1,000 mg/kg-d. Lesions in the forestomach were observed at 500 and 1,000 mg/kg-d groups along with effects observed in hematology and clinical chemistry parameters. The 90-day study groups were lowered to avoid irritation-based effects. In the 90-day study, no significant systemic toxicity effects were observed and a NOAEL of 225 mg/kg-d was established. Localized effects of forestomach irritation were observed at low and mid doses, establishing a 25 mg-d LOAEL for local effects. In a satellite group, reversal of the irritation effects only occurred in the 75 mg/kg-d group, but not the 25 mg/kg-d group. The 25 mg/kg-d group retained microscopic lesions of the forestomach.

5.3.3.1.2 Inhalation

Given that AES C12-14 2.5EO Na is a salt and will be present as a cation and anion in aqueous solution, it is unlikely to be vaporized. Aerosolized particles or aqueous suspended droplets/sprays are possible. The HERA Human Health Risk Assessment for AES (HERA 2003a) indicate inhalation exposure through powdered detergent and spray particles as the most likely route. Additionally, they report on a single inhalation study with rats exposed to AES C12-14 3EO NH_4 where 1 hour of exposure to 60 mg/L produced no mortalities. No other data were available (HERA 2003a).

A review of inhalation effects of cleaning products by Clausen et al. (2020) lists AES C12-14 2.5EO Na as a nonvolatile organic compound of unknown inhalation toxicity.

5.3.3.1.3 Dermal

As summarized in the HERA report, a large body of unpublished work demonstrates that dermal toxicity of AES C12 1EO Na (and AES as a class) is low (HERA 2003a). LD50s of AES in rats and rabbits are all above the highest dose in all studies—no mortality is observed in the rat studies and LD50s in rabbit with intact and abraded skin range from 4,000 – 12,000 mg/kg. A regulatory relevant dermal toxicity test in rats of AES C12-14 2EO NH_4 90% active ingredient produced an LD50 >2,000 mg/kg. Rats were dermally exposed to a single group for 24 hours and observed for 14 days. No systemic effects or mortality were observed. Importantly, moderate-to-severe inflammation/irritation of the skin was observed after the exposure period. After the 14-day observation period, only one animal had remaining lesions, all others improved (HERA 2003a).

Petersen (1988) exposed rabbits to two concentrations (23% and 27%) of AES C12-14 (no other chemical details were recovered) at 0, 0.5, 1.0, and 2.5% in distilled water for 6 hours/day, 5 days/week for 65 exposures across 91 days. No systemic effects were observed and only slight/transient irritation was observed. As indicated in the HERA report, skin is likely permeable to AES, so systemic exposure is likely given dermal exposure (HERA 2003a, Petersen 1988).

Importantly, irritation in skin by AES exposure has been determined to be concentration dependent (HERA 2003a, Robinson et al. 2010). In a series of (HERA 2003a, ECHA 2020g, Robinson et al. 2010), concentrations that represent very high exposures (~70%) are moderate-

to-severely irritating, high exposures (10 – 30%) are mild to moderately irritating, and low exposures (1%) are virtually nonirritating (HERA 2003a). The Cosmetic Ingredient Review (Robinson et al. 2010) indicates that there is potential for irritation to occur at concentrations present in cosmetic products.

Sensitization data summarized in the HERA report (largely unpublished data for ECHA registration) indicate that, AES C12 1EO Na is unlikely to be a skin sensitizer based on guinea pig exposures. The HERA report notes that some positive results (in non-OECD/non- GLP qualified studies) may be influenced by skin irritation. An OECD GLP qualified study of AES C12-14 2EO NH_4 required challenge patches at 25% active ingredient to avoid irritation observed in 50% active ingredient induction exposures (HERA 2003a).

5.3.3.1.4 Ocular

Ocular data available are largely concerned with irritation. Unpublished data summarized in the HERA report indicate that 28% AES C12-14 2EO Na is moderately to severely irritating to rabbits (HERA 2003a). In an OECD-compliant test irritation effects (corneal opacity) were still observed after 21 days.

An additional non-OECD/non-GLP study found that rinsing reduces the magnitude of ocular effects and recovery time after AES exposure and reduced concentrations (<1%) of AES are virtually nonirritating (HERA 2003a).

5.3.3.1.5 Development and Reproduction

A two-generation reproduction study following OECD and GLP guidelines summarized in the HERA report and ECHA registration dossier (HERA 2003a) indicates that AES C12-14 2EO Na (27% active ingredient) is not toxic to reproduction with a NOAEL of 300 mg/kg-d (the highest dose in the study). Rats (30/sex/dose) were exposed to 0, 0.03, 0.1, and 0.3% in drinking water, corresponding to 0, 30, 100, and 300 mg/kg-d. Slight and/or not systemic toxicological effects were observed in sperm motility, liver weights, triglyceride levels, and neutrophil counts. In offspring, increased time to sexual development was significant in females but not males. This effect was further investigated by dosing mated female rats with 100, 300, and 1,000 mg/kg-d and determined to be an anomaly. No embryotoxic or teratogenic effects were observed in offspring through maternal doses of 1,000 mg/kg-d (NOAEL) (HERA 2003a).

5.3.3.1.6 Genotoxicity

Exposure to AES C12-15 2-3EO Na in *E. coli*, *S. typhimurium*, *S. cerevisiae*, L5178Y TK+/- mouse lymphoma cells, rat liver cells, Syrian golden hamster embryo cells, and C3H 10T1/2 mouse embryo fibroblasts, with and without S9 activation produced negative assay results for genetic damage *in vitro* (HERA 2003a).

In vivo data, summarized in the HERA report (HERA 2003a), indicate additional negative mutagenic outcomes for AES. Specific to AES C12-15 2EO Na and AES C12-15 Ca, data indicate exposure in rats (single oral dose of 2.5 mL/kg for 6 hours) does not induce

deoxyribonucleic acid (DNA) strand damage recognized by the alkaline elution assay and that at 1.13% of diet for 90 days does not impact rat bone marrow cells (HERA 2003a, Hope 1977).

5.3.3.1.7 Carcinogenicity

The most specific study of AES carcinogenicity is summarized from unpublished data in the HERA report. AES C12 3EO was given to rats at 0.1% in drinking water for 2 years. There were no significant differences in tumors between the treated and control groups. Other effects attributed to advanced age were noted in both groups along with two potential treatment-related effects: increased water intake in treated animals and increased cecum: body weight in females (HERA 2003a).

5.3.3.1.8 Neurotoxicity

No data on the neurotoxic effects were found.

5.3.3.1.9 Mode/Mechanism of Action

Surfactants can be expected to interrupt cell walls and disrupt tissues (Falk 2019), which is corroborated by their irritation capacity. At a cellular level, ToxCast data for AES C12 1EO Na (no data available for AES C12-14 2.5EO Na) suggests activity with cell cycle interruption and nuclear receptor impacts below cytotoxic levels. Of the 77/438 active assays, 9 are below cytotoxic levels (CompTox 2020).

5.3.3.2 Ecological Data

5.3.3.2.1 Fate and Transport

Fate and transport of AES is likely to be defined by a short aerobic and anaerobic biodegradation half-life in aquatic systems (Cowan-Ellsberry et al. 2014; Scott and Jones 2000). Specific rates reported include a 7-day half-life in seawater and 17 days to 88% degradation in an anaerobic digester sludge (Cowan-Ellsberry et al. 2014). The HERA report and Scott and Jones (2000) summarize three environmentally relevant pathways in which the molecule can be cleaved and that result in no final metabolites which are more stable or toxic than the parent compound (HERA 2003b; Scott and Jones 2000). Caracciolo et al. (2019) report complete degradation of AES C12 1EO Na in soil by 28 days, with half lives of 6 and 8 – 9 days in silty-clay and clay-silty-sand matrix, respectively. These short half-lives and a log K_{ow} of 0.3 (ECHA 2020g), indicate low likelihood of bioconcentration or bioaccumulation.

Once the AES C12 1EO anion disassociates from the Na cation in water, the anionic component is generally considered hydrophobic. The level to which it is hydrophobic is a product of its carbon chain length and ethoxylation rate (HERA 2003b, Cowan-Ellsberry et al. 2014, Belanger et al. 2006, Scott and Jones 2000). AES C12-14 2.7EO have predicted aqueous solubility ranges from 425 to 41 mg/L (Cowan-Ellsberry et al. 2014). Estimated log K_{ow} parameters in this same group range from 0.95 to 1.9 (EPA 2013). Both of these values indicate that AES will be mobile in water until broken down. In contrast, reported data in the ECHA registration dossier indicates very high aquatic solubility and reduced log K_{ow} for AES C12-14

2.5EO Na; 280,000 mg/L via a flask and shaking, and log K_{ow} of 0.3 via a slow-stirring experimental method (ECHA 2020g). This suggests that while biodegradation is a dominant process, very mobile, high concentrations are possible. Based on the estimated log adsorption-desorption distribution coefficients for AES C12-14 2EO of 2.8-3.5, AES C12-14 2.5EO Na will likely move ~10^3 times slower than water (log K_d ~= 0, Belanger et al. 2006).

Given the ionic state of AES C12-14 2.5EO Na, atmospheric mobility is unlikely.

5.3.3.2.2 Ecotoxicity

The overarching ecotoxicological pattern demonstrated in representative aquatic invertebrate *C. dubia* is that as the number of ethoxy groups increases, toxicity decreases, and as the length of carbon chain increases, toxicity increases (Dyer et al. 2000). Acute 48-hour LC50 values in *C. dubia* relevant to this AES include 5.55, 1.58, 9.5, 55.98, 7.18, 4.08, and 4.24 mg/L corresponding to C12E0, C14E0, C12E1, C12E2, C13E2, C14E1, and C14E2 (Dyer et al. 2000). Chronic NOEC values for the same AES include 0.88, <0.06, 0.34, 6.25, 0.28, 0.34, and 0.31 mg/L. Chronic EC20 values for the same AES include 1.12, 0.23, 1.121, 17.38, 1.35, 1.05, and 0.37 mg/L. This dataset was used to fit a QSAR, which was applied in the HERA environmental risk assessment report (HERA 2003b; Dyer et al. 2000) and resulted in a PNEC for aquatic systems of 0.27, 0.076, and 0.038 mg/L for C12, C13, and C14, respectively, and include an uncertainty factor of 10.

In terrestrial systems, exposure is unlikely (ECHA 2020g). However, as demonstrated in Caracciolo et al. (2019), tunneling activities can release large quantities of AES to terrestrial environmental systems. Observations of reduced bacterial viability through time in exposed soils indicates potential for effects of AES exposure in soil systems, but appears to be transient and lasts less than 14 – 21 days (Caracciolo et al. 2019).

5.3.3.2.3 Degradation and Treatment

See paragraph 5.3.3.2.1for description of biodegradation, as this process likely dominates environmental processes relevant to AES. Biodegradation in WWTPs is nearly complete and occurs in both aerobic and anaerobic systems (Cowan-Ellsberry et al. 2014).

5.4 Carbohydrates

5.4.1 Sucrose [CASRN 57-50-1]

Sucrose is a white crystal or powder formed by the combining of a glucose and fructose and originates from sugar cane or sugar beet (NCBI 2020a). A common synonym for sucrose is saccharose. Figure 18 shows the structure of sucrose (CASRN 57-50-1).

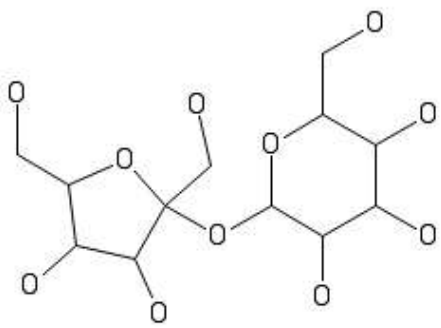

Figure 17. Structure of Sucrose (PubChem Sketcher 2021)

5.4.1.1 Toxicology Data

Sucrose is practically nontoxic with an oral lethal dose in humans likely above 15 g/kg (NCBI 2020a). There are some toxiciological considerations for sucrose as a dust, but when present in an aqueous suspension (such as in an AFFF), these considerations are null (NCBI 2020a).

5.4.1.2 Ecological Data

5.4.1.2.1 Fate and Transport

No data were found.

5.4.1.2.2 Ecotoxicity

Aquatic toxicity and toxicity to plants is low or negligible; no data for terrestrial species were found (EPA 2020b).

5.4.1.2.3 Degradation and Treatment

Sucrose is predicted to be readily biodegradable (EPA 2013).

5.4.2 Starch [CASRN 9005-25-8]

Starch is a fine, white powder that originates from various vegetable sources (NCBI 2020b). Common synonyms for starch include alpha-maltose, maltose, and amylodextrin. Starch is deemed GRAS by the U.S. FDA and is exempt from EPA TSCA reporting. Figure 19 shows the structure of starch (CASRN 9005-25-8).

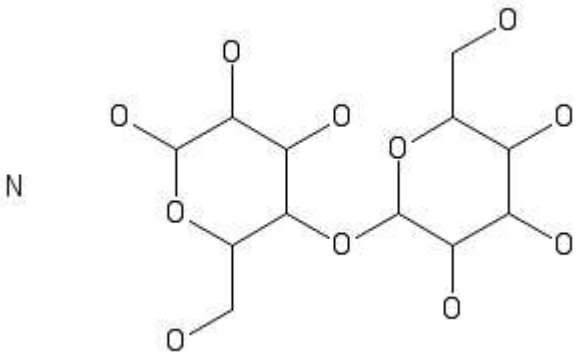

Figure 18. Structure of Starch (PubChem Sketcher 2021)

5.4.2.1 Toxicology Data

Starch is codified as generally low risk for health effects. There are some toxiciological considerations for starch as a dust, but when present in an aqueous suspension (such as in an AFFF), these considerations are null (NCBI 2020b).

5.4.2.2 Ecological Data

5.4.2.2.1 Fate and Transport

No data were found.

5.4.2.2.2 Ecotoxicity

Starch has been tested in various salt water fish and mollusk species at 3,000 – 5,000 mg/L with zero reported adverse effects (EPA 2020b).

Application of starch to soil produced a LOEL at 22.7 g starch/kg soil and NOEL at 2.27 g starch/kg soil in beans. A LOEL of 2.27 g starch/kg soil was found in corn (EPA 2020b).

5.4.2.2.3 Degradation and Treatment

No data were found.

5.5 Nonionic Surfactants

5.5.1 Glucopon 225DK (DG) [CASRN 68515-73-1]

The 2019 and 2021 NRL reports were not available at the time of preparation of this document.

Glucopon 225DK is a C8C10-alkyl polyglucoside as a 70% liquid (BASF 2018). It is identified as decyl glucoside (DG) (CASRN 68515-73-1). DG is a commonly used alkyl glucoside formed by the condensation of a decyl alcohol with a glucose (Loranger et al. 2017). The process to produce DG is considered “green” as the alcohols required for synthesis can come from coconut or palm oil; and wheat starch, corn, or potatoes can be used to obtain the glucose. DG can be monomeric or polymeric, and can be either a liquid or solid (Fiume et al. 2013). In liquid form, DG is a cloudy, yellow, viscous aqueous solution. This nonionic surfactant and cleansing agent is used in many over the counter items including skin lotions, soaps, shampoos, hair dyes, and sunscreen products (ChemicalBook 2020; Fiume et al. 2013). DG has also been used as a compound stabilizer for dermal delivery of nanosuspensions (Fiume et al. 2013, Kobierski et al. 2009).

Synonyms for DG include D-glucopyranose, decyl D-glucopyranoside, decyl glucopyranoside, capryl glycoside, and D-glucopyranose, oligomers, decyl octyl glycosides. Alkyl glucosides are named using the average side chain carbon length. On average, DG has a 10 carbon side chain. Figure 20 shows the structure of DG [CASRN 68515-73-1], the identified active ingredient in Glucopon 225DK.

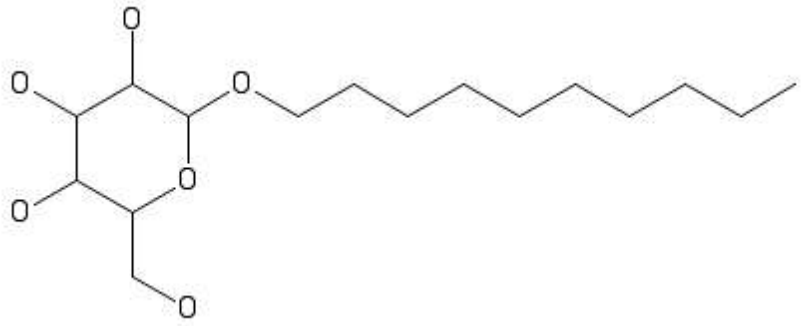

Figure 19. Structure of Decyl Glucoside (DG) (PubChem Sketcher 2021)

5.5.1.1 Toxicology Data

Decyl glucoside is a widely used nonionic surfactant that is listed within the EPA SCIL as a chemical verified to be of low concern (ChemView 2020). In 2013, the Cosmetic Ingredient Review Expert Panel reviewed 19 alkyl glucosides, including DG, and concluded that all 19 were safe to use if formulated to be nonirritating (Fiume et al. 2013).

5.5.1.1.1 Oral

The ECHA lists DG as practically nontoxic, with an oral LD50 in rats of 2,000 mg/kg. The oral repeated dose NOAEL for systemic toxicity in rat is 1,000 mg/kg-d (ECHA 2020i, Willing et al. 2004).

5.5.1.1.2 Inhalation

No reports for inhalation of DG were found. Based on the low vapor pressure of DG, it is likely not an inhalation hazard. However, DG is assigned the GHS code H304, indicating an aspiration hazard, and could be fatal if it enters the airways while being swallowed (NCBI 2020).

5.5.1.1.3 Dermal

Decyl glucoside is considered a skin irritant with assigned GHS codes H314 (Danger Skin corrosion/irritation) and H315 (Warning Skin corrosion/irritation) that can also cause skin burns at high enough concentrations (NCBI 2020).

Several studies were found for dermal exposure to DG to characterize irritation, allergenic, and sensitization properties. Decyl glucoside was found to be slightly irritating in a study of 20 patients using an epicutaneous patch test of a 2% active ingredient solution of DG at pH 6.5 (Fiume et al. 2013, Mehling et al. 2007). Slight irritation was also observed in 22 patients exposed to a 1% active ingredient DG at pH 6.5 using a soap chamber test (Fiume et al. 2013, Mehling et al. 2007). Human volunteers (N=100) were patch tested with 10% DG (aqueous solution) without demonstration of irritation (Loranger et al. 2017, Shanmugam et al. 2014). In addition, several case studies have been published describing reactions to DG-containing products (Andersen and Goossens 2006, Andrade et al. 2010, Blondeel 2003, Horn et al. 2005; Krehic and Avenel-Audran 2009, Le Coz and Meyer 2003). Follow on patch test studies demonstrated positive reactions to 0.5-10% DG (Fiume et al. 2013). Although these studies largely do not demonstrate DG-induced irritation, the DG concentrations tested were consistent with concentrations found in many commercial cosmetic products (0.002 – 8%) (Fiume et al. 2013).

Allergenic properties of glucosides were first described in 2003 with two case reports of lauryl glucoside and coco glucoside causing a contact allergic reaction (Goossens et al. 2003). Other studies followed, describing contact allergy from ingredients in products such as sunscreen, shampoo and other hair products, and an antiseptic (Blondeel 2003, Le Coz and Meyer 2003). A retrospective patch study of 897 patients with suspected cosmetic product-related dermatitis showed that 5% had a positive reaction to decyl or lauryl glucoside or both (Severin and Belsito 2017). A 10% aqueous DG solution is thought to be adequate for detecting allergic reactions without causing skin irritation (Alfalah et al. 2017, Blondeel 2003, Le Coz and Meyer 2003). In France, Belgium, and Switzerland, a 2% aqueous DG solution was added to the patch testing battery by the Groupe d' Etudes et de Recherches en Dermato-Allergologie (GERDA), and between 2005 and 2007, 0.5% of tested patients showed a positive reaction (Loranger et al. 2017). The North American Contact Dermatitis Group (NACDG) added 5% DG in petrolatum to the standard patch series in 2009 (Loranger et al. 2017).

Sensitization to alkyl glucosides found in sunscreens, cosmetics, and/or cleaning supplies can occur, but the mechanism driving sensitization is unknown. Four glucosides (decyl, lauryl, cetearyl, and coco) are responsible for most cases of sensitization, although alkyl glucosides of any length can cause sensitization (Loranger et al. 2017). However, four studies using aqueous concentrations of 0.5 – 5% (active ingredient) decyl glucoside have shown a lack of both dermal irritation and sensitization in a total of 362 patients (Fiume et al. 2013).

In addition to irritation and allergic and sensitization potential, alkyl glucosides may increase permeability of the skin and allow other compounds in a product that otherwise are not dermally absorbed to penetrate the dermal barrier (Fiume et al. 2013; Tirumalasetty and Eley 2006). For example, adding DG to into liposomes increased the skin permeability of caffeine (Abd et al. 2016).

5.5.1.1.4 Ocular

Decyl glucoside exposure can cause serious eye damage and irritation, depending on the concentration. It is assigned GHS codes H314 - causes severe skin burns and eye damage), H318 - causes serious eye damage, and H319 - causes serious eye irritation (NCBI 2020).

Three *in vitro* tests (rRBC test and HET-CM) and the Skinethic ocular tissue model) were used to predict ocular irritation potential of 0.6 – 3.0% (active ingredient) alkyl glucosides. At 1% DG in phosphate buffered saline (PBS) at pH 7 was not irritating in the RBC test. A 0.6% DG aqueous solution at pH 7 was shown to be nonirritating in the Skinethic ocular tissue model. Finally, a 3% aqueous DG solution at pH 6.5 was predicted to be slightly irritating in the HET-CM test (Fiume et al. 2013, Mehling et al. 2007).

5.5.1.1.5 Development and Reproduction

Although no studies were found for DG, a development and reproduction study with lauryl glucoside (12-carbon chain) was found. Oral exposure to lauryl glucoside in rats did not yield any reproductive or developmental effects and did not produce maternal toxicity with doses up to 1,000 mg/kg-d from GD6 – 15. The NOAEL was set at 1,000 mg/kg-d for teratogenicity, embryotoxicity, and maternal toxicity. This study also demonstrated this NOAEL in a second cohort of rats (up to 1,000 mg/kg-d for 2 weeks prior to mating and up to 4 days post-delivery). Results from this cohort demonstrated no effects on male or female sex organs, on any reproductive parameter, or on litter or pup weights, pup sex ratio, or gestational timeframe (Messinger et al. 2007).

5.5.1.1.6 Genotoxicity

The Ames test was used to determine the mutagenic potential of unspecified alkyl glucosides using a concentration ranges of either 8 – 500 mg/L or 11 – 900 mg/plate. Regardless of metabolic activation, the alkyl glucosides did not display any mutagenicity. In another study, C10-16 alkyl glucosides were evaluated for clastogenicity using Chinese hamster V79 lung fibroblasts. The C10-C16 alkyl glucosides were assayed for chromosomal aberrations at ≤ 160 mg/mL (metabolic activation) and ≤ 16 mg/mL (no metabolic activation). No clastogenicity was observed (Fiume et al. 2013, Willing et al. 2004).

5.5.1.1.7 Carcinogenicity

No reports were found.

5.5.1.1.8 Neurotoxicity

No reports were found.

5.5.1.1.9 Mode/Mechanism of Action

The mechanism for dermal irritation is unknown. Dermal metabolism of alkyl glucosides likely occurs, with glucoside hydrolases present in human skin breaking down the alkyl glucoside into glucose and the corresponding fatty alcohol (Mueller and Rosenberg 1977).

5.5.1.2 Ecological Data

5.5.1.2.1 Fate and Transport

Decyl glucoside is very soluble in water and has a log K_{ow} of 1.92 (ChemSpider 2020). Decyl glucoside is a nonvolatile liquid with a low vapor pressure (≤ 7.5 x 10-6 atm-m^3/mol; (ChemSpider 2020; ECHA 2020i). The calculated log K_{oc} for DG is 1.0 (ChemSpider 2020), indicating negligible soil adsorption. Presence of DG is not expected to affect the environmental fate and transport of other compounds (Carretta et al. 2020, Cederlund and Börjesson 2016). For example, DG in the presence of the herbicide glyphosate did not significantly change the environmental fate of the pesticide (Carretta et al. 2020). The BCF for DG is estimated as 5.961 (ChemSpider 2020). Based on these properties, DG is not a bioaccumulation concern (CompTox 2020).

5.5.1.2.2 Ecotoxicity

Decyl glucoside is considered a moderate hazard for both acute and chronic aquatic toxicity (ChemView 2020) and is assigned the GHS code H412 for category 3 chronic aquatic toxicity (harmful to aquatic life with long lasting effects), but has no degradants of concern (ECHA 2020i). The 4-day LC50 for fish is 100.81 – 170 mg/L, with an LC50 for freshwater fish of 100.81 mg/mL and marine water fish of 96.64 mg/mL. The 28-day NOEC for fish is 1 – 3.2 mg/L and the 28-day LC50 is 3.2 mg/L (ECHA 2020i). Nonionic decyl glucoside showed low to no toxicity in zebrafish at concentrations up to 200 ppm (~200 mg/L) (Han and Jung 2020).

Decyl glucoside has been shown to inhibit sea urchin egg first cleavage in a dose-dependent manner, but the IC50 was >400 mg/mL. The effect was the greatest at 20 – 50 minutes post-fertilization. The mechanism for this inhibition is thought to involve intracellular pH and DNA synthesis (Amouroux et al. 1999).

Decyl glucoside is reported to have a 48-hour EC50 and NOEC of 100 mg/L for aquatic invertebrates. The 21-day NOEC for aquatic invertebrates is 1 – 4 mg/L with a LOEC of 2 – 4 mg/L (ECHA 2020).

Decyl glucoside toxicity values for aquatic algae and cyanobacteria have been reported, with a 72-hour EC50 range of 27.22 – 37 mg/L. The low end of that range corresponds to the EC50 for freshwater algae. Marine water algae have a lower EC50 for DG at 7.03 mg/L. The NOEC for freshwater algae has been reported as 6.25 mg/L DG (ECHA 2020i).

Data were not found for DG toxicity to terrestrial plants or birds. However, sediment toxicity was reported with a 10-day NOEC range of 262.16 – 436.93 mg/kg sediment (dry weight) and a 10-day LC50 range of 3,318.81 – 5,531.35 mg/kg (ECHA 2020i).

5.5.1.2.3 Degradation and Treatment

Decyl glucoside is readily biodegradable (ECHA 2020i) and is considered a very low hazard for environmental persistence (ChemView 2020). The sewage treatment plant PNEC on aquatic organisms is 560 mg/L (ECHA 2020i).

5.6 Unidentified

5.6.1 Preservative [CASRN not available]

The specific preservative ingredient is not disclosed by the manufacturer.

5.6.1.1 Toxicology Data

5.6.1.1.1 Oral

Acute oral toxicity GHS classification 3 (BIOEX 2017).

5.6.1.1.2 Inhalation

Acute inhalation toxicity GHS classification 3 (BIOEX 2017).

5.6.1.1.3 Dermal

Acute dermal toxicity GHS classification 3, skin corrosion GHS category 1B, skin sensitization GHS category 1 (BIOEX 2017).

5.6.1.1.4 Ocular

No data were found.

5.6.1.1.5 Development and Reproduction

No data were found.

5.6.1.1.6 Genotoxicity

No data were found.

5.6.1.1.7 Carcinogenicity

No data were found.

5.6.1.1.8 Neurotoxicity

No data were found.

5.6.1.1.9 Mode/Mechanism of Action

No data were found.

5.6.1.2 Ecological Data

5.6.1.2.1 Fate and Transport

No data were found.

5.6.1.2.2 Ecotoxicity

Acutely toxic to aquatic life, GHS category 1. The LC50 in *O. mykiss* is 0.19 mg/L, the EC50 in *D. magna* is 0.16 mg/L, and the EC50 in *Scenedesmus capricornutum* is 0.027 mg/L (BIOEX 2017).

5.6.1.2.3 Degradation and Treatment

No data were found.

5.7 Summaries

Individual constituent physical and chemical properties are summarized in Table 2. Individual constituent toxicity is summarized in Table 3. Individual constituent ecotoxicity data are summarized in Table 2.

Table 2. Individual Constituent Physical and Chemical Properties

Compound	CASRN	Molar Mass (g/mol)	Melting Point (ºC)	Boiling Point (ºC)	Aqueous solubility (mg/L) @ 20ºC	log K_{ow}	log K_{oc}	Henry's Law Constant @ 25ºC (atm-m^3/mol)	Vapor Pressure mmHg @ 25°C
Amphiphilic Solvents									
DGBE [a]	112-34-5	162.23	-68.11	231.11	955,000	0.56	10	15.2e-09	0.0219
HG [b]	107-41-5	118.17	-50.0	197.0-198.0	Miscible-68,780	0.58	1	4.06X10-7	0.05-0.07
Amphoteric Surfactants									
Amphoteric Surfactant	not disclosed								
CAPHS C12 [c]	68139-30-0	424.64	333.26	280.5	520,000	0.61	1.9963	$2.90e^{-29}$	$<3.2^{-3}$
CAPHS C8-18 [d]	ECN 939-455-3	ND	61.4	280.5	556 g/L @20°C	2.1 @ 25°C	129.42 @20°C	ND	0
Alkylbetaine	not disclosed								
AES C9-11 1-3EO Na [e]	96130-61-9	C9 = 365.2 C10 = 379.3 C11 = 393.4	ND	ND	C9= 14,211 C10= 4410 C11= 1369	C9= -0.56 C10= -0.06 C11= 0.43	ND	ND	C9=1.2e-14 C10=5.2e-15 C11=2.2e-15
CAPB C12 [f]	61789-40-0	342.5	283	651	23,676	-1.28 – 2.69	1.531-2.811	3.90^{-22}	4.81^{-15}
AO C12 [g]	1643-20-5	229.4	132.5	335	190,000[a]	4.67	3.443	6.6e-11	1.86e-6
AO C14 [h]	3332-27-2	257.462	127	Decomposes	0.32 exp 0.01 obs	5.66 exp 4.48 5.73 obs	3.15 obs	1.05e-5 obs	6.07e-3 obs 1.11e-8 exp
AO C12-14 [i]	308062-28-4	237	125-134	Decomposes before boiling	409.5 g/L @20°C	<2.7 @ 20°C -1.08	1525 L/kg @ 20°C	3.7e-09 – 1.2e-08 Pa.m3/mol	<4.6e-7 hPa 1.6e-06 – 7.5e-05 hPa
PDMS EO [j]	67674-67-3	398.79	71.86	>110 335.6	0.01357	7.40	4.22-4.57	9.54e-5	4.84e-6
AS C12 TEA [k]	139-96-8	415.59	72-122.5	708.6	Miscible,844.5	2.55	3.1	9.5e-21	9.9e-22
AS C12-14 TEA [l]	90583-18-9	415-443	300.6-310.06,	708.6, 688.39 Decomposes	844.5,5.8	0.55,3.0	3.3-1.0	9.5e-21,4.6e-19	9.9e-22,3.5e-20
Anionic Surfactants									
Alkylsulfate	not disclosed								
AS C12 Na [m]	151-21-3	288.38	253.95 206	588.52	100,000-616.8 0.2 mol/L	1.6 1.69	1.972	1.84e-7 2.33e-7	1.8e-12 1.2e-3 4.7e-13
AES C12-14 2.5EO Na [n]	68891-38-3	332.43	>300	>400	280,000	0.3	log Kd=~3	7.03e-8	1.51e-15
Carbohydrates									
Sucrose [o]	57-50-1	342.4	decomposes	decomposes	500,000	-3.70	ND	0	0
Starch [p]	9005-25-8	342.3	decomposes	decomposes	0	ND	ND	ND	0

Compound	CASRN	Molar Mass (g/mol)	Melting Point (ºC)	Boiling Point (ºC)	Aqueous solubility (mg/L) @ 20ºC	log K_{OW}	log K_{OC}	Henry's Law Constant @ 25ºC (atm-m^3/mol)	Vapor Pressure mmHg @ 25°C
Nonionic Surfactants									
DG [q]	68515-73-1	320.42; 340.2	NA or 300 or 135.6	100 or 300 or 467.5	Soluble; 215; 58-200	1.92	1.0	2.0×10^{-13}	$7.5 - 60 \times 10^{-6}$; 4.35×10^{-12}
Unidentified									
Preservative	not disclosed								

Legend:
ND = no data
NA = not applicable
Notes:
Experimental and predicted values are mixed in this table.
502W additive contains Si, a metalloid that may or may not be accurately represented by the models in EPI Suite.
Multiple entries or ranges indicate multiple sources or sources present range of values.
[a] ECHA 2011, NCBI 2020a
[b] CAMEO Chemicals 2020, ECHA 2020a, NCBI 2020b, SIDS 2001, Verevkin 2007
[c] CIR 2017, ECHA 2020c, NCBI 2020e, EPA 2013
[d] CIR 2017
[e] Cowan-Ellsberry et al. 2014, HERA 2003b
[f] ECHA 2020b, Könnecker et al. 2011, NCBI 2020d, EPA 2013
[g] NCBI 2020f, EPA 2018, ECHA 2020i
[h] ECHA 2021b
[i] ECHA 2020d, Kirk-Othmer 2001, Mlynarcik et al. 1985, SIDS 2006, The Procter & Gamble Co. 2002, TSCA 1983
[j] EPA 2013
[k] Könnecker et al. 2011, EPA 2013
[l] Könnecker et al. 2011, The Procter & Gamble Co. 2002, EPA 2013, OPERA 2020
[m] CompTox 2020, NCBI 2020g, Singer and Tjeerdema 1993, EPA 2013
[n] Belanger et al. 2006, ECHA 2020g, ECHA 2020h, NCBI 2020h, EPA 2020a
[o] NCBI 2020i
[p] NCBI 2020j
[q] BASF 2018, ChemSpider 2020, ECHA 2020i, Fiume et al. 2013, Goossens et al. 2003, PubChem Sketcher 2021

Table 3. Individual Constituent Toxicity Data

Compound	CASRN	Acute Oral (mg/kg)	Chronic Oral LOAEL (mg/kg-d)	Inhalation (g/m^3-h)	Dermal	Ocular	Development/ Reproduction (mg/kg-d)	Geno-toxicity	Carcino-genicity
Amphiphilic Solvents									
DGBE [a]	112-34-5	5660	1500	>10	Positive irritant; negative sensitizer	Irritant	NOAEL = 1,000	Negative	Negative
HG [b]	107-41-5	>2,000-4,700	1.5	7.8 mg/m^3	Low potential	Slightly irritating	$LOAEL_{mat}$ = 1,000 $NOAEL_{fet, mat}$ = 300	Negative	ND
Amphoteric Surfactants									
Amphoteric Surfactant	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA
CAPHS C12 [c]	68139-30-0	3020 (24hrs) 2950 (14 d)	ND	ND	Not a skin irritant or dermal sensitizer	Severe eye irritant	ND	Negative	ND
CAPHS C8-18 [c]	ECN 939-455-3	2,950	ND	ND, but estimated to be low	Negative sensitizer and irritant	Moderate to severe irritant	300 mg/kg-d NOEL for devel. and repro.	Not mutagenic	ND
Alkylbetaine	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA
AES C9-11 1-3EO Na [d]	96130-61-9	2,000	>250	>60	Irritant	Negative	Irritant	NOAEL>1000 mg/kg -d[a]; NOAEL > 300 mg/kg -d	Neg
CAPB C12 [e]	61789-40-0	5,000	ND	ND	Skin irritant	Eye irritant	NOAEL 1,000 mg/kg	Negative	ND
AO C12 [f]	1643-20-5	1,064	50	5.3	Skin irritant	Irreversible irritant	NOAEL = 25	Negative	Negative
AO C14 [g]	3332-27-2	>1,500	440	1	Irritant, no sensitization	Irritation	NOAEL = 25 mg/kg-d	Negative	Negative by read-across
AO C12-14 [h]	308062-28-4	1,064	88 NOAEL	No studies available	Skin irritant > 2000, irritation reversible	Reversible irritant	NOAEL ≥ 37-128 mg/kg-d	Low	Low
PDMS EO [i]	67674-67-3	>5,050	ND	2.3*	ND	Irreversible	ND	Negative	ND
AS C12 TEA [j]	139-96-8	>2,000	ND	0.135 mg/L	Irritant, dose dependent (moderate at high, low at low)	Severe Irritant	ND, see text for read-across	Negative	ND, see text for read-across

Compound	CASRN	Acute Oral (mg/kg)	Chronic Oral LOAEL (mg/kg-d)	Inhalation (g/m^3-h)	Dermal	Ocular	Development/ Reproduction (mg/kg-d)	Geno-toxicity	Carcino-genicity
AS C12-14 TEA [k]	90583-18-9	1000-2000	306	0.135 mg/L 50% reduction in respiration rate	Irritant, dose dependent (severe at high, low and low)	Severe Irritant	ND, see text for read-across	Negative	ND, see text for read-across
Anionic Surfactants									
Alkylsulfate	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA
AS C12 Na [l]	151-21-3	1,288	500	ND	Mod irritation	Severe irritation	ND	negative	negative
AES C12-14 2.5EO Na [m]	68891-38-3	1,995	500	>60	Irritant	Irritant	>300/>1000 mg/kg	Negative	Negative
Carbohydrates									
Sucrose	57-50-1	GRAS	GRAS	GRAS	GRAS	GRAS	GRAS	GRAS	GRAS
Starch	9005-25-8	GRAS	GRAS	GRAS	GRAS	GRAS	GRAS	GRAS	GRAS
Nonionic Surfactants									
DG [n]	68515-73-1	2,000 (rat, rabbit); >5,000 (rat)	ND	NA	Skin Irritant	Irritant	ND	Negative	ND
Unidentified									
Preservative	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA

Legend:
ND = no data NA = not applicable GRAS = Generally Recognized as Safe
Notes:
*dusts & mists
Experimental and predicted values are mixed in this table.
Multiple entries or ranges indicate multiple sources or sources present range of values.
[a] BIOVA 2015, Gollapudi et al. 1993, NCBI 2020a, Sitarek et al. 2012, Thompson et al. 1984, EPA 2009
[b] CIR 1985, ECHA 2020a, Guillot et al. 1982, Kinnunen and Hannuksela 1989, SIDS 2001
[c] CIR 2017, WCHA 2020c
[d] HERA 2003a, Little 1991
[e] ECHA 2020b, FDRL 1982, Goldschmidt Chemical Corp 1993b, Goldschmidt Chemical Corp 1993c, Henkel 1986c, Henkel 1987b, HERA 2005, Th. Goldschmidt AG 1990b, Th. Goldschmidt AG 1991b, EPA 1993
[f] DSSTOX 2020, ECHA 2020e, NCBI 2020f
[g] ECHA 2021b, NCBI 2020c
[h] ECHA 2020d, Haferkorn 2010, SIDS 2006, The Procter & Gamble Co 1979a, The Procter & Gamble Co 1979b
[i] Snow 2016
[j] ECHA 2021d
[k] Ciuchta and Dodd 1978, ECHA 2021c, HERA 2002a, Little 1991, SIDS 2007, Wibbertman et al. 2011
[l] Lewis 2004, NCBI 2020g, SIDS 2007, Walker et al. 1967
[m] ECHA 2020g, HERA 2003a, Hope 1977, Walker et al. 1967
[n] BASF 2018, ECHA 2020i, Fiume et al. 2013, NCBI 2020k

Table 4. Individual Constituent Ecotoxicity Data

Compound	CASRN	Modeled				Experimental			
		Model Class	Green Algae EC50 (mg/L)	Daphnia LC50 (mg/L)	Fish LC50 (mg/L)	Green Algae EC50 (mg/L)	Daphnia LC50 (mg/L)	Fish LC50 (mg/L)	Other
Amphiphilic Solvents									
DGBE	112-34-5	Neutral organics	857	2.21E3	4.56E3	ND	ND	ND	ND
HG [a]	107-41-5	NA	NA	NA	NA	>429	3200; 5410	8510-12800 (multiple species)	ND
Amphoteric Surfactants									
Amphoteric Surfactant	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA
CAPHS C12 [b]	68139-30-0	Anionic Surfactant, C12 (ECOSAR)	0.0072 (ECOSAR)	2.6 (ECOSAR)	2.6 (ECOSAR)	ND	ND	ND	ND
CAPHS C8-18 [c]	ECN 939-455-3	NA	NA	NA	NA	2.69	1.39 Chronic NOEC	> 0.27-2.66	ND
Alkylbetaine	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA
AES C9-11 1-3EO Na [d]	96130-61-9	Anionic Surfactant	C9=3.38 C10=0.300 C11=0.039	C9=924 C10=91.3 C11=12.9	C9=924 C10=91.3 C11=12.9	ND	C9AE2=275 C10AE2=136 C11AE2=67.4	ND	PNEC C<12AE2.7= >0.27 mg/L
CAPB C12 [e]	61789-40-0	Amphoteric surfactant (C18 + 1 ethoxylate)	0.056 (ECOSAR)	0.056 (ECOSAR)	0.056 (ECOSAR)	ND	ND	ND	ND
AO C12 [f]	1643-20-5	Aliphatic amine (ECOSAR)	0.056 (ECOSAR)	0.126 (ECOSAR) 7.6e-9 (TOPKAT)	0.773 (ECOSAR) 0.0208 (TOPKAT)	0.266	ND	31.8 (zebrafish)	HC5 = 0.052 mg/L
AO C14 [g]	3332-27-2	Cationic surfactant C<16 (ECOSAR)	NA (ECOSAR)	1.778 (ECOSAR)	1.778 (ECOSAR)	0.19	2.64	2.4	ND
AO C12-14 [h]	308062-28-4	NA	NA	NA	NA	0.143 0.014	3.1	3.13, 2.67-3.46, 1.7, 3.5, 12.6, 3-30	ND
PDMS EO [i]	67674-67-3	Neutral organic (ECOSAR)	NA (ECOSAR)	ChV = 1.69e-3 (ECOSAR)	ChV = 8.13e-4 (ECOSAR)	ND	>1-10	>1-10	ND
		Amphoteric surfactant (ECOSAR 2.0)	0.667, NES (ECOSAR 2.0)	0.667, NES (ECOSAR 2.0)	0.667, NES (ECOSAR 2.0)				
AS C12 TEA [j]	139-96-8	Amides -acid	1131.124 (ECOSAR)	1.93e5 (ECOSAR)	59558.418 (ECOSAR)	ND	ND	ND	ND
		Alkyl-Nitrogen-Ethoxylates	13.932 (ECOSAR)	13.932 (ECOSAR)	13.932 (ECOSAR)				

Compound	CASRN	Modeled				Experimental			
		Model Class	Green Algae EC50 (mg/L)	Daphnia LC50 (mg/L)	Fish LC50 (mg/L)	Green Algae EC50 (mg/L)	Daphnia LC50 (mg/L)	Fish LC50 (mg/L)	Other
AS C12-14 TEA [k]	90583-18-9	Anionic surfactant	C12=0.0072 C14=0.00078 (ECOSAR)	C12=2.6 C14=0.306 (ECOSAR)	C12=2.6 C14-0.306 (ECOSAR)	ND	38	9.2	ND
Anionic Surfactants									
Alkylsulfate	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA
AS C12 Na [l]	151-21-3	Neutral organic (ECOSAR)	47 (ECOSAR)	54 (ECOSAR)	91 (ECOSAR)	ND	ND	ND	ND
AES C12-14 2.5EO Na [l]	68891-38-3	Anionic surfactant	C12=0.0072 C13=0.002 C14=0.00078 (ECOSAR)	C12=2.6 C13=0.746 C14=0.306 (ECOSAR)	C12=2.6 C13=0.746 C14=0.306 (ECOSAR)	ND	ND	ND	PNECC12=0.27 C13=0.076 C14=0.038
Carbohydrates									
Sucrose	57-50-1	NA	NA	NA	NA	>5060	ND	>60000	ND
Starch	9005-25-8	NA	NA	NA	NA	ND	ND	>5000	ND
Nonionic Surfactants									
DG	68515-73-1	Neutral Organics	1.02×10^4	3.66×10^4	8.17×10^4	ND	ND	ND	ND
Unidentified									
Preservative	not disclosed	NA	NA	NA	NA	NA	NA	NA	NA

Legend:
ND = no data NA = not applicable ChV = chronic value (geometric mean of NOEC and LOEC) NES = No Effect at Saturation
Notes:
Experimental and predicted values are mixed in this table.
502W additive contains Si, a metalloid that may or may not be accurately represented by the models in EPI Suite.
Multiple entries or ranges indicate multiple sources or source(s) present range of values.
[a] Brooke et al. 1984, Elnabarawy et al. 1986, ECHA 2020a, SIDS 2001, Thiebaud and Chedaille 1999, Thurston et al. 1985
[b] EPA 2020a
[c] ECHA 2021a
[d] Dyer et al. 2000, EPA 2017
[e] EPA 2020a
[f] Belanger et al. 2016, ECHA 2020e, EPA 2020a, Belanger et al. 2006
[g] ECHA 2020f, SIDS 2006, EPA 2020a
[h] Dommrose and Grutzmacher 1987, ECHA 2020d, Hoechst 1987, Macek and Sleight 1977, Noack 2001, SIDS 2006
[i] Snow 2016, EPA 2020a
[j] EPA 2020a
[k] SIDS 2007, EPA 2020a
[l] EPA 2020a

6. UNCERTAINTY ANALYSIS

Sources of uncertainty dominate the assessment of hazard or toxicity in situations of limited data at product and/or constituent level (See Table 5). Quantifying sources of uncertainty formalizes the identification of sources of uncertainty as well as allows for ease of objective downstream interpretation.

The ability to identify constituents (i) is largely associated with inclusion of a CASRN on SDS. While quantifying identification rate aids in capturing the impact of identification uncertainty, using class-based narratives can provide some mitigation (paragraph **Error! Reference source not found.** in main report). In some cases with high quality identification information (such as a CASRN), no data was recovered on search (ii). While predictions may address these true data gaps, there were cases when this wasn't possible and "ND" categorizations are listed for certain endpoints and result in < 1 completeness rates (See Table 5). To capture the impact of predictions filling data gaps, the proportion of endpoint categories derived from experimental data is another factor in the data quality score (iii). The sum of the three proportions (i-iii) representing identification rate, completeness rate, and experimental data rate is the data quality score. The data quality score scales from 0 to 3 with a high score of 3 indicating complete identification data, complete endpoints represented in literature search, and a complete proportion of experimental data. The last source of uncertainty is considered by the authors to be the dominant uncertainty impacting the assessment of toxicity/hazard of these products. The final quantity produced in the uncertainty analysis—the trustworthiness score—is the data quality score weighted by the maximum percent of the product makeup disclosed in the SDS (iv). Accordingly, this trustworthiness score can also range from 0 to 3 with a 3 representing the maximum trust in data used for categorization by the analyst.

At the constituent-level, data quality scores are calculated for each constituent within each product. Then to provide a product-level value, the median constituent-level data quality score is weighted by the percent of the concentrate disclosed in SDS. This approach is based on a constituent-based literature search prior to the acquisition of product-level testing data. These values are presented in detail in Table 5.

Table 5. Uncertainty Table of AFFF Replacements Based on Information Contained in Section 7.3[a]

Product	Maximum % known	Data Quality Score	Trustworthiness Score
BIOEX ECOPOL A 3%	32.5	0.32	0.10
Fomtec ENVIRO USP	23.0	2.65	0.61
National Foam 20-391	24.0	2.71	0.65
National Foam AvioF3 Green KHC 3%	61.0	2.74	1.67
NRL 502W	33.7	2.65	0.89
Solberg Re-Healing Foam RF3 3%	74.5	2.49	1.85

7. GENERAL CONSTITUENT HAZARD SUMMARIES

Table 6 and Table 7 include ‘stoplight’ interpretation of constituent-level data using GHS and Howe et al. (2007) categories. These are included as reference for the individual constituents and present summarized endpoint categories from the full ‘stoplight’ interpretive matrices in the main document.

Table 6. Individual Constituent Toxicity Assessment

Compound	CASRN	Oral	Inhalation	Dermal	Ocular	Carcinogenicity	Comments
Amphiphilic Solvents							
DGBE	112-34-5	Low	Low	Low	Mod	Low	
HG	107-41-5	Low	Mod	Low	Mod	Unk	
Amphoteric Surfactants							
Amphoteric Surfactant	not disclosed	Mod	Unk	Low	Mod	Low	Assumed; based on class
CAPHS C12	68139-30-0	Low	Unk	Low	High	Unk	
CAPHS C8-18	ECN 939-455-3	Low	Unk	Low	Mod	Unk	
Alkylbetaine	not disclosed	Mod	Unk	High	High	Unk	Assumed; based on class
AES C9-11 1-3EO Na	96130-61-9	Low	Low	Mod	Mod	Low	Ocular data based on read-across
CAPB C12	61789-40-0	Low	Unk	Low	Mod	Unk	
AO C12	1643-20-5	Low	Low	High	High	Low	
AO C14	3332-27-2	Mod	Mod	Mod	High	Low†	
AO C12-14	308062-28-4	Mod	Unk	Low	Mod	Low	
PDMS EO	67674-67-3	Low	Mod	Unk	High	Unk	Modeled or from SDS
AS C12 TEA	139-96-8	Low	Mod‡	Low-Mod	Low-Mod	Unk	
AS C12-14 TEA	90583-18-9	Mod	Mod	High	High	Unk	Dermal/ocular toxicity decreases w/ concentration
Anionic Surfactants							
Alkylsulfate	not disclosed	Low-Mod	Unk	Mod	High	Low	Assumed; based on class
AS C12 Na	151-21-3	Mod	Unk	High	High	Low	
AES C12-14 2.5EO Na	68891-38-3	Low	Low‡	Mod	High	Low	
Carbohydrates							
Sucrose	57-50-1	Low	Low	Low	Low	Low	GRAS
Starch	9005-25-8	Low	Low	Low	Low	Low	GRAS
Nonionic Surfactants							
Glucopon 225DK (D-Glucopyranose, oligomers, dedyl octyl glycosides, 60-100%)	68515-73-1	Low	Low	High	High	Unk	
Unidentified							
Preservative	not disclosed	Mod	Mod	High	Unk	Unk	All data from SDS

Notes:
†based on read-across
‡dust/mist

Table 7. Individual Constituent Ecotoxicity Assessment

Compound	CASRN	Aquatic	Invertebrates	Plants	Mammals	Birds	Comments
Amphiphilic Solvents							
DGBE	112-34-5	Low	Low	Unk	Low	Unk	
HG	107-41-5	Low	Low	Low	Low	Unk	
Amphoteric Surfactants							
Amphoteric Surfactant	not disclosed	High	Unk	Unk	Low-Mod	Unk	Assumed; based on class
CAPHS C12	68139-30-0	Low	Unk	Low	High	Unk	
CAPHS C8-18	ECN 939-455-3	Low-Mod	Unk	Unk	Unk	Unk	
Alkylbetaine	not disclosed	Unk	Unk	Unk	Unk	Unk	
AES C9-11 1-3EO Na	96130-61-9	Mod	Unk	Unk	Unk	Unk	PNEC is Cat 1, but biodegradation is high
CAPB C12	61789-40-0	High	High	Unk	Unk	Unk	predicted
AO C12	1643-20-5	High	Unk	Unk	Unk	Unk	
AO C14	3332-27-2	High	Mod	High	Unk	Unk	
AO C12-14	308062-28-4	Mod	Low	Unk	Unk	Unk	
PDMS EO	67674-67-3	High	Unk	Unk	Unk	Unk	Aquatic toxicity is chronic only
AS C12 TEA	139-96-8	Mod	Mod	Unk	Unk	Unk	
AS C12-14 TEA	90583-18-9	Mod	Mod	Unk	Unk	Unk	
Anionic Surfactants	not disclosed						
Alkylsulfate	not disclosed	Mod	Unk	Unk	Unk	Unk	Assumed; based on class
AS C12 Na	151-21-3	Mod	Mod	Low	Unk	Unk	
AES C12-14 2.5EO Na	68891-38-3	High	Unk	Unk	Unk	Unk	
Carbohydrates							
Sucrose	57-50-1	Low	Unk	Low	Unk	Unk	
Starch	9005-25-8	Low	Unk	Low	Unk	Unk	
Nonionic Surfactants							
Glucopon 225DK (D-Glucopyranose, oligomers, dedyl octyl glycosides, 60-100%)	68515-73-1	Low-Mod	Low	Unk	Low	Unk	
Unidentified							
Preservative	not disclosed	High	Unk	Unk	Unk	Unk	Data from SDS

APPENDIX A
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