2-ETHYLHEXYL ACRYLATE (2-EHA)

(CAS #103-11-7)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: November 16, 2021

Expiration Date: November 16, 2026

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GreenScreen® Executive Summary for 2-Ethylhexyl Acrylate (2-EHA) (CAS #103-11-7)

2-Ethylhexyl acrylate, also called 2-EHA, is an acrylic monomer that is commonly used to manufacture polymers for acrylic adhesives. It is also used as a binding agent in cosmetic formulations. 2-EHA is a colorless liquid that is slightly soluble in water. It is a combustible liquid and may undergo spontaneous polymerization when exposed to sunlight or heat.

2-EHA was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes"). This score is based on the following hazard score:

- Benchmark 2e
    - Moderate Group I Human Toxicity (carcinogenicity-C)

Data gaps (DG) exist for endocrine activity-E and neurotoxicity repeated dose-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), 2-EHA meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if 2-EHA were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity, endocrine activity, skin irritation, and skin sensitization and *in silico* models for respiratory sensitization and endocrine activity. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

Type I (input data) uncertainties in EHA's NAMs dataset include the absence of experimental data and established test methods for endocrine activity and respiratory sensitization. 2-EHA's Type II (extrapolation output) uncertainties include the limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, the limitation of the *in vitro* skin corrosion test (OECD Guidelines 431) to identify substances classified as skin irritants (GHS Category 2), the limitation of the *in vitro* skin sensitization assays to address chemicals that are pre-haptens, the unknown *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* modeling of receptor binding, and the lack of defined applicability domains in OECD Toolbox as well as ToxCast models. Some of the type I and type II errors can be alleviated by the use of genotoxicity test batteries, *in vivo* data for skin irritation and sensitization and ECHA's decision framework and guidance to evaluate respiratory sensitization.

GreenScreen® Hazard Summary Table for 2-EHA

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
M	**L**	*L*	*L*	DG	**L**	**M**	*M*	*M*	DG	**M**	*M*	**H**	**L**	**H**	*H*	**L**	**L**	*L*	**M**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Chemical Assessment for 2-Ethylhexyl Acrylate (2-EHA) (CAS #103-11-7)

Method Version: GreenScreen® Version 1.4
Assessment Type[1]: Certified
Assessor Type: Licensed GreenScreen® Profiler

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Mouna Zachary, Ph.D.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: August 18, 2021, November 16, 2021

Quality Control Performed By:
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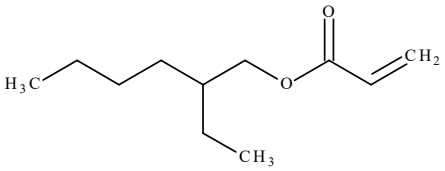
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: August 18, 2021, November 16, 2021

Expiration Date: August 18, 2026[2]

Chemical Name: 2-Ethylhexyl acrylate (2-EHA)

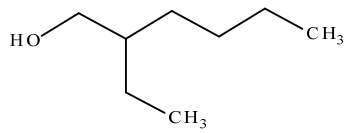
CAS Number: 103-11-7

Chemical Structure(s):



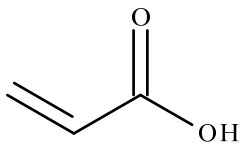
Also called: 1-Hexanol, 2-ethyl-, acrylate, 2-Ethyl-1-hexyl acrylate, 2-Ethylhexyl 2-propenoate, 2-Propenoic acid, 2-ethylhexyl ester, Acrylic acid, 2-ethylhexyl ester, Mono(2-ethylhexyl) acrylate (ChemIDplus 2021).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
2-EHA has a relatively complete toxicological dataset. For the reproductive and developmental toxicity endpoints, the available study on 2-EHA which was conducted according to OECD Guideline 422 and GLP was considered of low reliability by the authors of its REACH dossier due to methodology deficiencies. Therefore, data on its two primary metabolites/hydrolysis products, 2-ethylhexanol (2-EH) (CAS #104-76-7) and acrylic acid (CAS #79-10-7), were considered**.**



Surrogate: 2-EH (CAS #104-76-7)

[1] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[2] Assessments expire five years from the date of completion starting from January 1, 2019. An assessment expires three years from the date of completion if completed before January 1, 2019 (CPA 2018a).



Surrogate: Acrylic acid (CAS #79-10-7)

Identify Applications/Functional Uses: (Pharos 2021)
1. Monomer for plastics, protective coatings, paper treatment; in water-based paints.
2. Monomer in vinyl acetate copolymerization.
3. Binding agent in cosmetic formulations.

Known Impurities[3]:
2-EHA is available as a commercial product with a purity of 99% or greater. Impurities/additives include: water, at 0.05–0.10 %, acidity (as acrylic acid) at 0.009 % (maximum); hydroquinone (polymerization inhibitor) at 90–120 ppm; and monomethyl ether of hydroquinone (polymerization inhibitor) at 13–120 ppm (IARC 2019).

GreenScreen® Summary Rating for2-EHA[4,5 6,7]: 2-EHA was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes") (CPA 2018b). This score is based on the following hazard score:

- Benchmark 2e
    - Moderate Group I Human Toxicity (carcinogenicity-C)

Data gaps (DG) exist for endocrine activity-E and neurotoxicity repeated dose-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis) (CPA 2018b), 2-EHA meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if 2-EHA were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for 2-EHA

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
M	**L**	*L*	*L*	DG	**L**	**M**	*M*	*M*	DG	**M**	*M*	**H**	**L**	**H**	*H*	**L**	**L**	*L*	**M**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of

[3] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[4] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.
[5] See Appendix A for a glossary of hazard endpoint acronyms.
[6] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[7] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen® Guidance v1.4 Annex 2.

repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

Environmental Transformation Products

Per GreenScreen® guidance (CPA 2018b), chemicals that degrade rapidly and completely (i.e., meet criteria for a Very Low for persistence) are not likely to form persistent biodegradation intermediates because the degradation intermediates will not persist long enough to be encountered after use or release of the parent chemical (i.e., relevant). As 2-EHAis rapidly biodegradable, it is not expected to have relevant transformation products.

Introduction

2-Ethylhexyl acrylate, also called 2-EHA, is an acrylic monomer that is commonly used to manufacture polymers for acrylic adhesives. It is manufactured by direct, acid-catalyzed esterification of acrylic acid with 2-ethylhexanol. The monomethyl ether of hydroquinone is added as a polymerization inhibitor, and the esters are used in this form in most industrial applications (IARC 2019).

ToxServices assessed 2-EHA against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2020).

U.S. EPA Safer Choice Program's Safer Chemical Ingredients List (SCP SCIL)

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2021a). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

2-EHA is not the not listed on the SCP SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2021) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[8] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for 2-EHA can be found in Appendix C.

- 2-EHA is an LT-UNK chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- 2-EHA is not listed on the U.S. DOT list.
- 2-EHA is on the following lists for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.
    - German FEA - Substances Hazardous to Waters - Class 1 - Low Hazard to Waters
    - Quebec CSST – WHMIS 1988: Class D2B – Toxic material causing other toxic effects

[8] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

Hazard Statement and Occupational Control

2-EHA is associated with several European Union (EU) harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements as shown in Table 1, reported by the European Chemicals Agency (ECHA 2021a). General personal protective equipment (PPE) recommendations are presented in Table 2 below. A small number of countries have occupational exposure limits (OELs) for 2-EHA as shown in Table 2.

Table 1: GHS H Statements for 2-EHA (CAS #103-11-7) (ECHA 2021a)	
H Statement	**H Statement Details**
H315	Causes skin irritation (EU- GHS, harmonized)
H317	May cause an allergic skin reaction (EU- GHS, harmonized)
H335	May cause respiratory irritation (EU- GHS, harmonized)

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for 2-EHA (CAS #103-11-7)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Wear protective gloves/protective clothing/eye protection/face protection.	CAMEO 2021	TWA: 8h = 38 mg/m^3	IARC 2019
TWA: Time Weighted Average			

Physicochemical Properties of 2-EHA

2-EHA is a colorless, clear liquid under standard temperature and pressure. Its measured vapor pressure (24 Pa) indicates that it exists mostly as a vapor in the ambient atmosphere. It is slightly soluble in water (9.6 mg/L). Its log K_{ow} value (4.64) indicates a slight potential to bioaccumulate.

Table 3: Physical and Chemical Properties of 2-EHA (CAS #103-11-7)		
Property	**Value**	**Reference**
Molecular formula	$C_{11}H_{20}O_2$	ChemIDplus 2021
SMILES Notation	CCCCC(CC)COC(=O)C=C	ChemIDplus 2021
Molecular weight	184.277	ChemIDplus 2021
Physical state	Liquid	ECHA 2021b
Appearance	Colorless / Transparent	ECHA 2021b
Melting point	-90°C (data from handbook or collection of data)	ECHA 2021b
Boiling point	215°C (data from handbook or collection of data)	ECHA 2021b
Vapor pressure	24 Pa at 25°C (data from handbook or collection of data)	ECHA 2021b
Water solubility	9.6 mg/L at 25°C (EU Method A.6)	ECHA 2021b
Dissociation constant	Not applicable as the substance does not contain any ionic structure.	ECHA 2021b
Density/specific gravity	0.88 at 20°C	ECHA 2021b

Table 3: Physical and Chemical Properties of 2-EHA (CAS #103-11-7)		
Property	**Value**	**Reference**
Partition coefficient	Log K_{ow} = 4.64 at 25°C (similar to OECD Guideline 107)	ECHA 2021b

Toxicokinetics

- Absorption and Excretion.
  - ECHA 2021b
    - *Oral*: Absorption of 2-EHA by oral route of exposure is expected as excretion of a radiolabeled dose was seen in rats administered a single dose of 2-EHA by gavage. In this study, two groups of 3 male F344/DuCrl rats were exposed to either radioactive 14C-2-EHA or its hydrolysis product 14C-2-EH via gavage at equal molar dose levels and equal molar levels of radioactivity (100 or 70.6 mg/kg of 2-EHA and 2-EH, respectively). The excretions in urine, feces and air (volatiles and CO_2) were determined. The total average recovery (0-168 hours) was 94% and 96 %, respectively. The recoveries (0-24 h) from urine, feces and CO_2 trapping solution were 56, 18 and 11% for 14C-2-EHA and 63, 16 and 9% for 14C-2-EH, respectively (Klimisch 1, reliable without restriction).
    - *Oral: Surrogate 2-EH (CAS #104-76-7)*: 2-EH at high doses has been demonstrated to undergo saturation of metabolism following oral bolus dosing. Excretion balance studies were conducted with 2-EH in female Fischer 344 rats following single high (500 mg/kg) and low (50 mg/kg) oral doses of [14C]-2-EH, following repeated oral dosing with unlabeled 2-EH at the low level, and following a 1 mg/kg i.v. dose of [14C]-2-EH. The high, low and repeated low oral dose studies with 2-EH showed similar excretion balance profiles of [14C], with some evidence of metabolic saturation at the high dose. No evidence of metabolic induction was seen following the repeated low oral dosing. All of the oral doses were eliminated rapidly, predominantly in the urine during the first 24 h following dosing. Urinary metabolites eliminated were predominately glucuronides of oxidized metabolites of 2-EH, including glucuronides of 2-ethyladipic acid, 2-ethylhexanoic acid, 5-hydroxy-2-ethylhexanoic acid and 6-hydroxy-2-ethylhexanoic acid.
      - Based on above, the authors of REACH dossier assumed a default oral absorption rate of 100% for 2-EHA.
    - *Inhalation*: Absorption of 2-EHA by inhalation is expected as urinary excretion of metabolites was observed in rats exposed to air concentrations of 2-EHA ranging from 250 mg/m^3 to 4,800 mg/m^3. When male Wistar rats were exposed for 6 hours by inhalation to 2-EHA at concentrations from 250 to 4,800 mg/m^3 over 24 hours, excreted thioethers were seen in the urine in a dose dependent manner decreasing from 8.0 to 3.0% (at 1,000 mg/m^3) of the dose of 2-EHA, indicating saturable metabolism along this pathway. Dose related depletion of non-protein SH groups in blood, liver and brain was seen at concentrations of and above 2,400 mg/m^3. Based on this as well as the physicochemical properties of 2-EHA (lipophilic compound (log P >4) with a low water solubility (9.6 mg/L) and it may be taken up by micellular solubilization), authors of its REACH dossier assumed a default inhalation absorption rate of 100%.
    - *Dermal*: No measured data were available. Authors of REACH dossier estimated the rate of absorption of 2-EHA using the IH SkinPerm model (v2.04). For an

instantaneous deposition of 1,000 mg over 1,000 cm² of skin or a deposition rate over time of 1 mg/cm²/h, the absorption rates were calculated to be 1.8% and 0.2% of the dose after 8 hours of contact, respectively. Based on this, REACH dossier authors assumed a dermal absorption fraction of 10% for 2-EHA.

- Distribution
  - ECHA 2021b
    - *Intraperitoneal*: In two basic toxicokinetics studies, male Wistar rats (24) were administrated an intraperitoneal dose of either 10 mg/kg of (14C)-2-EHA labelled on the vinyl carbons or 100 mg/kg of [2,3-14C]-2-EHA in soybean oil. The test animals were sacrificed between 0 and 72 hours after administration and subjected to necropsy. The following organs were isolated and the amount of radioactivity determined: liver, kidneys, spleen, lungs, brain, and a sample of the sciatic nerves and of the fatty tissue. In both studies plasma radioactivity concentration reached a peak level at about 2-3 hours after administration indicating easy absorption through this route. In tissues the highest concentrations of radioactivity were found in kidney, liver, spleen and the lungs. In the study with a dose of 100 mg/kg, 6.5% of the dose was found in tissues at 3 hours after administration. The radioactivity in the tissues decreased slowly with time. At 72 hours after administration 1% of the dose was still found in the examined tissues. The radioactivity in adipose tissue and sciatic nerve was still relatively high (Klimisch 2, reliable with restrictions).
    - *Intravenous*: In one study male Wistar rats were administrated an intravenous dose of 10 mg/kg of (14C)-2-EHA labelled on the vinyl carbons. In another study male Wistar rats were administrated an intravenous dose of 10 mg/kg or 50 mg/kg of (14C)-2-EHA. The highest concentrations of radioactivity in tissues were found in kidney, liver, brain, thymus and spleen (Klimisch 2, reliable with restrictions).
- Metabolism
  - ECHA 2021b
    - The major route of metabolism of acrylate esters, including 2-EHA, involves the rapid cleavage of the ester bond by carboxylic esterases, resulting in internal exposure to acrylic acid (AA) and the corresponding alcohol. A subsequent metabolic pathway involves metabolism of AA to carbon dioxide (CO_2) via the propionate degradation pathway. The respective alcohols are metabolized via either a catalase peroxidation pathway or the alcohol dehydrogenase pathway. Acrylate esters are also expected to undergo conjugation with GSH to form thioesters with the main urinary conjugate identified as N-acetyl-S-(2-carboxyethyl)cysteine. Inhibition of the hydrolytic pathway with a carboxylase inhibitor results in increased metabolism via the GSH conjugation route. There is no evidence to suggest that the vinyl moiety undergoes epoxidation. Based on a recent in vitro investigation for the hydrolysis and glutathione conjugation rates of the acrylate esters, 2-EHA and the majority of other acrylates tested were metabolized by rat liver microsomes in the presence or absence of ß-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) to form AA. It was reported that the hydrolysis of the acrylate esters in rat liver microsomes is mainly mediated by esterases, which does not require NADPH. A summary of the in vitro and in vivo metabolism assays for 2-EHA are described below
      - *In vitro*: In an *in vitro* assay performed to evaluate the hydrolysis properties of acrylate esters including 2-EHA using liver S9 fraction and blood plasma of male Wistar rats, the decrease of the acrylates and the formation of acrylic

acid was determined analytically. In liver S9 fraction, the degradation of acrylates and the formation of acrylic acid was clearly observed. The half-life was 1.15 min for 2-EHA and the degradation rate was 5.165 µM/g liver equivalent*min. In plasma, the degradation times were slower than in liver S9 fraction. The half-life was 6.48 min for 2-EHA and the degradation rate was 59.86 µmol/L*min (Klimisch 2, reliable with restrictions).

- *In vitro*: The hydrolysis of selected acrylate esters including 2-EHA was also investigated in another *in vitro* assay using rat liver microsomes and whole rat blood at a single substrate concentration of 500 µM. The incubation was performed in combination with the presence or absence of microsomes and NADPH. All acrylate esters except tert-butyl acrylate (tert-BA) were metabolized by rat liver microsomes in the presence or absence of NADPH to form AA. Without microsomes, all acrylate esters were relatively stable under the incubation conditions, indicating the hydrolysis of acrylate esters was mainly catalyzed by the enzymes contained in rat liver microsomes. The concentrations of the remaining acrylate esters, both measured concentrations and the back-calculated concentrations from the formation of AA, support the similarity between the microsomal incubations regardless of the presence of NADPH. This suggests that the hydrolysis of acrylate esters in rat liver microsomes is mainly due to the esterases which do not require NADPH for the enzymatic hydrolysis of acrylate esters. The tested acrylate esters (methyl acrylate (MA), ethyl acrylate (EA), butyl acrylate (BA), iso-butyl acrylate (iso-BA) and 2-EHA) have a half-life of less than 8.5 minutes (0.77-8.2 min) in the rat liver microsomes, indicating that metabolism is rapid. tert-BA was relatively stable under the same microsomal incubation conditions, probably due to the presence of steric hindrance due to its tertiary structure. The time-course of the remaining acrylate esters, both measured and back-calculated values, showed a rapid metabolism of the acrylate esters with almost complete consumption of the acrylate esters within the culture. However, the concentrations of the formed AA were significantly lower in the rat blood compared to the microsomal culture. The half-lives for all acrylate esters, based on the measured concentrations of the remaining parent acrylate esters, were less than 12 minutes in rat blood, ranging 0.99 – 11.2 minutes (Klimisch 1, reliable without restriction).
- *In vivo*: The metabolism of 2-EHA was also investigated in the previously described study in which two groups of 3 male F344/DuCrl rats were exposed to either radioactive 14C-2-EHA or its hydrolysis product 14C-2-EH via gavage at equal molar dose levels and equal molar levels of radioactivity (100 or 70.6 mg/kg of 2-EHA and 2-EH, respectively). The blood samples were collected at Cmax (0.17 hour), 1/2Cmax (1 hour) and 1/5Cmax (12 hour) and representative blood samples were profiled. Similar metabolite profiles were observed. No detectable 14C-2-EHA levels were found in any blood samples. 14C-2-EH was the only major metabolite observed in all Cmax or 1/2Cmax blood samples. These results support a common metabolic pathway with 2-EH after gavage administration of 2-EHA or 2-EH in rats (Klimisch 1, reliable without restriction).
- *In vivo: Surrogate 2-EH (CAS #104-76-7)*: In the previously described excretion balance studies conducted with 2-EH in female Fischer 344 rats

following single high (500 mg/kg) and low (50 mg/kg) oral doses of [14C]-2-EH, following repeated oral dosing with unlabeled 2-EH at the low level, and following a 1 mg/kg i.v. dose of [14C]-2-EH, urinary metabolites eliminated were predominately glucuronides of oxidized metabolites of 2-EH, including glucuronides of 2-ethyladipic acid, 2-ethylhexanoic acid, 5-hydroxy-2-ethylhexanoic acid and 6-hydroxy-2-ethylhexanoic acid.

- In summary, absorption of 2-EHA by oral and inhalation route of exposure is expected with a default absorption rate of 100%. Assumed absorption for the dermal route is low (10%). Following oral, intravenous and intraperitoneal administration in rats, 2-EHA is extensively distributed, and the highest concentrations were found in kidney, liver, brain, thymus and spleen. 2-EHA is expected to undergo rapid ester hydrolysis to form 2-EH and acrylic acid. The main excretion pathway is urine.

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): M

2-EHA was assigned a score of Moderate for carcinogenicity based on being classified to Group 2B – (Possibly carcinogenic to humans) by the IARC authoritative list supported by positive results in skin painting studies. GreenScreen® criteria classify chemicals as a Moderate hazard for carcinogenicity when they are classified to Group 2B – (Possibly carcinogenic to humans) by the IARC authoritative list. The confidence in the score is high as it is based on an authoritative list.

- Authoritative and Screening Lists
  - *Authoritative:* IARC - Group 2B - Possibly carcinogenic to humans
  - *Screening:* Not present on any screening lists for this endpoint.
- IARC 2019
  - 2-EHA was tested for carcinogenicity in three skin application studies in male mice. In two studies in C3H/HeJ mice, 2-EHA caused a significant increase in the incidence of squamous cell papilloma and of squamous cell papilloma or carcinoma (combined) of the skin in one study, and a significant increase in the incidence of papilloma, cornified squamous cell carcinoma, malignant melanoma, and of fibrosarcoma of the skin in the second study. In the third study, which used a different strain of mice, 2-EHA did not significantly increase the incidence of tumors of the skin either with or without subsequent ap application of 12-O-tetradecanoylphorbol-13-acetate. Based on this, the International Agency for Research on Cancer (IARC) Working Group concluded that there is sufficient evidence in experimental animals for the carcinogenicity of 2-EHA and classified it to Group 2B - Possibly carcinogenic to humans. A summary for each study is provided below.
    - *Dermal:* In an early dermal life-time carcinogenicity study, 40 male C3H/HeJ mice were treated dermally with a 75% solution of 2-EHA (99% purity) in acetone 3 times/week at an average dose of 20 µg 2-EHA/application (approximately 750 mg/kg /day according to IARC). All mice exposed to 2-EHA were dead 2 years after the start of the experiment. No information on body weights or other clinical observations was reported. Treatment caused neoplastic skin lesions in six animals. Four males had a statistically significant increase in the incidence of squamous cell papilloma of the skin (10%) and two others had squamous cell carcinomas (15%). The authors concluded that 2-EHA is carcinogenic in C3H mice. Based on this, the IARC working group concluded that 2-EHA induced skin tumors only at concentrations exceeding the maximum tolerated dose (MTD) and only in the

immune-dysregulated C3H/HeJ mouse model. In addition, although the study was limited because of the use of only one sex and a single dose, and a limited dosing for only 3 days per week, the IARC Working Group considered it was still performed adequately according to the standards of that time for skin application studies for an evaluation of the carcinogenicity of 2-EHA. *Authors of REACH dossier considered this study not reliable (Klimisch 3, not reliable) due to major methodological deficiencies (ECHA 2021b).*

- *Dermal:* In a more recent dermal life-time carcinogenicity study, male C3H/HeJ mice (80/dose) were exposed to a 25-µL solution of 2-EHA (≥ 99.5% purity) in acetone at 0% (vehicle control), 2.5%, 21%, or 86.5% (equivalent to 24.8, 212, and 937 mg/kg/day according to IARC) to the clipped dorsal skin three times per week for their lifetime. Another group was treated with a 43% 2-EHA solution (approx. 444 mg/kg/day); for 24 weeks and thereafter observed for lifetime (stop-test). Animals were evaluated for body weight, clinical symptoms, and skin irritation. Gross lesions and the dorsal skin were fixed. The skin tissue from the application site was the only tissue that was examined histologically. Treatment caused scaling and scabbing in all exposed groups and persisted throughout the treatment period. Regression of these skin lesions was observed within 7 weeks after stopping treatment in the stop-exposure group. Treatment also caused a statistically significant increase in the incidence of papilloma of the skin in 4 males at highest dose group. In addition, statistically significant increase in the incidence of cornified squamous cell carcinoma of the skin and of malignant melanoma was observed for groups exposed at the intermediate and highest doses. Five mice developed fibrosarcoma of the skin and one mouse developed a basal cell carcinoma of the skin in the group exposed at the intermediate dose, and one hemangioma of the skin was observed in the group exposed at the highest dose. No skin tumors were reported in the control (untreated or vehicle) groups, the group exposed at the lowest dose, or the stop-exposure group. Based on this, the IARC working group concluded that 2-EHA induced skin tumors only at concentrations exceeding the MTD and only in the immune-dysregulated C3H/HeJ mouse model. In addition, although this study may have used higher concentrations than recommended by current guidelines, it was conducted according to the contemporary standards of that time and in a widely used and accepted strain of mouse for skin application studies. Although the study was limited because of the use of only one sex and limited dosing for only 3 days per week, the IARC Working Group considered it in the evaluation of the carcinogenicity of 2-EHA as it was still performed adequately according to the standards of that time for skin application studies. *Authors of REACH dossier concluded that irritative skin lesions were precursors of the neoplasia and assigned a LOAEL for local nonneoplastic effects on the skin of 24.8 mg/kg/day, the lowest dose tested. The study was reported in the REACH dossier of 2-EHA with a Klimisch reliability score of 3 (not reliable) due to major methodological deficiencies (ECHA 2021b).*
- *Dermal:* In a skin painting study, male NMRI mice exposed were dermally exposed to 25 µl of 21.5%, 43%, or 85% 2-EHA (99% purity) diluted in acetone (approximately 269, 538 and 1,063 mg/kg/day as calculated by the study authors) 3 times/week for 7 months. Exposure to 2-EHA was discontinued at 7 months, and after 2 months mice were exposed to a solution of 12-O-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL acetone, at a dose of 5 µg per mouse twice per week for 20 weeks, and observed for up to an additional 10 months. Body weights and

survival were similar between exposed and control animals. One squamous cell papilloma of the skin was seen at the application site in the groups exposed to 2-EHA (lower, intermediate, and higher doses) plus TPA; no squamous cell carcinomas or keratoacanthomas of the skin were reported in these groups. No tumors of the skin were observed in the acetone plus TPA control group. A positive control group of mice exposed to benzo[a]pyrene plus TPA developed squamous cell carcinomas or keratoacanthomas of the skin. The IARC Working Group noted that the study was limited by the use of only one sex, the limited dosing of only 3 days per week, the provision of data and discussion of histopathology for the skin only, and the lack of detailed information on survival and body weight. *The study was reported in the REACH dossier of 2-EHA with a Klimisch reliability score of 2 (reliable with restrictions) (ECHA 2021b).*

- ECHA 2021b
    - Findings from the mouse dermal carcinogenicity studies showed that 2-EHA induces skin tumors at concentrations which were highly irritative. It was concluded that tumor growth is associated with the highly irritative properties of 2-EHA. At a low concentration of 2.5%, at which 2-EHA caused transient irritation, no tumor response of the skin was observed. Other long-term studies on different mouse strains did not confirm tumor induction of the mouse skin. Additionally, there is no concern from tumor data on acrylic acid and 2-ethylhexanol, the hydrolysis products of 2-EHA.
    - Taking into account the negative results from *in vivo* genotoxicity testing, it is concluded that 2-EHA induces skin tumors by a non-genotoxic mechanism. Irritative skin damage was identified as presumed mode of tumorigenicity associated with carcinogenic effect of 2-EHA. Due to the limited reliability of skin painting studies in mice as a tool to identify the carcinogenic potential of a test substance these studies give some concern but no clear evidence that 2-EHA has carcinogenic potential. Based on limited database from dermal studies and absence of carcinogenicity data for the oral and inhalation routes, no conclusion could be drawn about the carcinogenic potential of 2-EHA. However, taking into account the negative experimental results from long term animal studies with the cleavage product acrylic acid after oral and dermal application, there are no reasons to assume that 2-EHA should be considered as a carcinogenic substance. In addition, based on recent publications the skin painting studies in C3H/HeJ mice using 2-EHA have to be regarded as non-reliable. The C3H/HeJ mouse model is not appropriate as it has a mutation in Toll-like receptor 4 (TLR4) that impairs its innate and adaptive immune responses. Inconsistencies in the histological evaluation of tumors induced in C3H/HeJ mice provide further evidence that the tumorigenic effect of 2-EHA was strain specific, a result of chronic inflammation during the promotion stage and/or a skewed immune response caused by the TLR4 mutation.
- Based on the weight of evidence, a score of Moderate was assigned. Findings from the dermal mouse carcinogenicity studies showed that 2-EHA induces skin tumors at concentrations which were highly irritative. Irritative skin damage was identified as presumed mode of tumorigenicity. Authors of REACH dossier considered these studies not reliable as the C3H/HeJ mouse model used is not the appropriate model. Further, the authors of REACH dossier concluded that 2-EHA should not be considered as a carcinogenic substance based on negative experimental results from long term animal studies with the cleavage product acrylic acid after oral and dermal application. However, ToxServices relied on the recent classification by the IARC authoritative list (Possibly carcinogenic to humans) and assigned a score of Moderate.

Mutagenicity/Genotoxicity (M) Score (H, M, or L): L

2-EHA was assigned a score of Low for mutagenicity/genotoxicity based on negative results for mutagenicity and clastogenicity observed in a battery of *in vitro* and *in vivo* studies. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative results for mutagenicity and clastogenicity and no GHS classification are available (CPA 2018). The confidence in the score is high as it is based on the weight of evidence from multiple *in vitro* and *in vivo* studies for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
  - *In vitro*: 2-EHA (purity unspecified) was negative in a bacterial mutagenicity assay that was conducted in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 tested at concentrations of 3.3 – 10,000 µg/plate with and without metabolic activation. There were no increases in revertants in any strain at any dose, and positive and vehicle controls were valid (Klimisch 2, reliable with restrictions).
  - *In vitro*: 2-EHA (purity unspecified) was negative in a bacterial mutagenicity assay that was conducted in *S. typhimurium* strains TA 1535, TA 100, TA 97, TA 98 tested at concentrations of 0.3 – 200 µg/plate with and without metabolic activation. There were no increases in revertants in any strain at any dose, and positive and vehicle controls were valid (Klimisch 2, reliable with restrictions).
  - *In vitro*: 2-EHA was negative for mutagenicity in a GLP-compliant *in vitro* mammalian cell mutagenicity assay that was conducted according to OECD Guideline 476. V79 cells of the Chinese hamster were exposed to the test material at 14.4-230 µg/ml, with and without metabolic activation. No increase in the mutation frequency at the HPRT locus was observed in the presence or absence of metabolic activation. The vehicle and positive controls were valid (Klimisch 1, reliable without restriction).
  - *In vitro*: 2-EHA yielded equivocal results in an *in vitro* mammalian gene mutation test in which mouse lymphoma cells were exposed to the test substance at doses of 20, 25, 31 and 34 µg/ml for 4 hours without metabolic activation. The mutant frequency at thymidine kinase (Tk) locus was increased at some test doses; however, the mutant frequency was not increased at higher concentrations and was not consistent across trials. In addition, cell survival was lower than 50%. All treatments resulted in strong cytotoxicity (27, 16, 12 and 12 % relative survival) (Klimisch 2, reliable with restrictions).
  - *In vitro*: Negative results for clastogenicity were obtained in a GLP-compliant *in vitro* mammalian cell micronucleus test conducted according to OECD Guideline 487. Human lymphocytes cells were exposed to 2-EHA in ethanol or acetone at concentrations of 8.4 to 1,843 µg/mL for 4 or 20 hours with and without metabolic activation. There were no increases in the numbers of micronucleated cells. The vehicle and positive controls were valid (Klimisch 1, reliable without restriction).
  - *In vivo*: In a GLP-compliant unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells conducted according to OECD Guideline 486, male Wistar rats were administered 2-EHA (99.9% purity) in corn oil by gavage at single doses of 1,000 and 2,000 mg/kg. Hepatocytes were harvested 3 and 14 hours after administration of the test substance. As a negative control, male rats were administered merely the vehicle, corn oil, by the same route, which gave frequencies of mean nuclear net grain counts within the historical control range. The positive control chemical 2-acetylaminofluorene (2-AAF) administered once orally in a dose of 50 mg/kg body weight demonstrated the expected

increase in unscheduled DNA synthesis. On the basis of the results from the present study, the single oral treatment with the test substance did not lead to an increase in the mean number of net nuclear grain counts at any dose level or exposure time in rat hepatocytes. Study authors concluded that 2-EHA is considered to be negative in the *in vivo* UDS assay using rat hepatocytes (Klimisch 1, reliable without restriction).

Reproductive Toxicity (R) Score (H, M, or L): *L*

2-EHA was assigned a score of Low for reproductive toxicity based on the lack of reproductive effects in reproductive toxicity studies conducted with its hydrolysis products, acrylic acid and 2-ethyhexanol. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate negative data are available, and they are not GHS classified (CPA 2018b). Although the score is based on measured data of high quality for strong surrogates and a conclusion from an authoritative body for the surrogate 2-ethyhexanol, the confidence in the score is reduced due to some effects observed in the unreliable OECD 422 study on 2EA, and an OECD Guideline 443 (Extended One-Generation Reproductive Toxicity Study) study on the target chemical being underway; the results from this study will take precedence over surrogate data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
  - *Oral*: In a GLP-compliant repeated dose toxicity study with a reproductive and developmental toxicity screening study conducted according to OECD Guideline 422, Sprague-Dawley rats, (10/sex/dose) were administered 2-EHA (99.518% purity) via gavage at doses of 0, 75, 250, 750 or 1,000 mg/kg/day. Males were treated for a total of 28 days beginning 14 days prior to mating and through sacrifice on day 28. Females were exposed for 41-55 days, i.e., during 2 weeks prior to mating, during mating, during post-coitum, and until day 13 of lactation. The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, clinical chemistry, reproductive performance (male or female mating index, male or female fertility index, male copulation index, number of corpora lutea, number of implantations), gross pathology, and histopathology. Offspring were evaluated for survival, mean litter size, sex ratio, body weight, and external and internal abnormalities. Treatment caused parental systemic effects in males at doses from 250 mg/kg/day and females at 750 mg/kg/day as characterized by clinical observations and microscopic changes in the stomach and liver. No effects indicative of F0 male reproductive toxicity were noted at any dosage level tested. However, test substance-related effects on gestation length, implantation sites, number of pups born, and litter size were noted at 750 and 1,000 mg/kg/day. Therefore, authors assigned a NOAEL of 250 mg/kg/day for female reproductive toxicity. Authors of REACH dossier considered this study not reliable (Klimisch 4, not assignable) because analytical verification of the dose formulations showed large variabilities (33 - 131 %) and several dosing formulations did not meet the acceptability criteria. Therefore, the relevance of the findings is not clear. Accordingly, data on its two hydrolysis products, 2-EH and acrylic acid, were considered**.**
- ECHA 2021b,c
  - *Oral*: <u>*Surrogate: Acrylic acid (CAS #79-10-7):*</u> In a GLP-compliant two-generation reproduction toxicity study conducted according to OECD Guideline 416, Wistar rats (25/sex/dose) received the test substance (98.9% purity) in their drinking water at doses of 0, 53, 249, or 460 mg/kg/day. Treatment caused no adverse effects in the male F_0 generation. In the female F_0 generation there was reduced food and water consumption in the 460

mg/kg/day group during pregnancy and a dose-dependent decrease in food and water consumption during lactation in the 240 mg/kg/day group. There was a dose-dependent decrease in food and water consumption and a concomitant decrease in body weight and weight gain in both sexes in the F_1 generation at 240 mg/kg/day. Treatment had no adverse effects on fertility, pre-implantation development, or reproductive organs. Treatment did not alter the male mating index in either generation. The authors identified a NOAEL for reproduction function of 460 mg/kg/day (highest dose tested). The NOAEL for general toxicity was 240 mg/kg/day for the F0 generation, but 53 mg/kg/day for the F1 generation (Klimisch 1, reliable without restriction).

- *Oral*: *Surrogate: Acrylic acid (CAS #79-10-7):* In a one-generation reproductive toxicity study similar to OECD Guideline 415, F334/N rats (10 males and 20 females per group) received doses of 0, 83, 250, or 750 mg/kg/day test substance in drinking water for 13 weeks. One male rat was mated with two females and exposure continued for both sexes throughout gestation and lactation. Treatment caused a dose-dependent decrease in food and water consumption and consequently body weight in the F_0 animals. Exposure to 750 mg/kg/day caused decreases in fertility index in males and females, the gestation index, the number of pups born alive, and the percentage of pups weaned. Pups in the 750 mg/kg/day group had decreased body weight gain, a reduction in absolute and absolute liver weights in males, and a reduction in both absolute and relative spleen weights in females. The European Commission (EC) noted that these findings were not considered an indication of any substantial deleterious effect of acrylic acid on reproductive performance because there were no statistically significant differences between the treated and control groups (EC 2002). However, it was noted that the fertility index and litter size of the control group in this study was uncharacteristically low (Klimisch 2, reliable with restrictions).

- ECHA 2015, 2021b,
  - *Surrogate: 2-EH (CAS #104-76-7):* ECHA evaluated the reproductive toxicity of 2-EH and concluded it is not a reproductive toxicant based on the results of a two-generation reproductive toxicity study with its precursor di (2 -ethylhexyl) terephthalate (DEHT) together with various supporting studies for 2-EH which demonstrate the lack of toxicity to reproduction (e.g., no adverse effect on any gestational parameter in prenatal developmental studies, no effect on testes and ovaries of rats and mice in 90-day repeated dose gavage studies, no anti-androgenic activity *in vitro* or degeneration of testes (*in vivo*) and Sertoli cells (*in vivo* and *in vitro)*). The highest dose tested in the two-generation study with DEHT is in the range of the maximum tolerable dose (MTD) for 2-EH as required by the testing guideline. ECHA also reviewed the reproductive toxicity data of 2-ethylhexanoic acid (ECHA 2017a), which is the major urinary metabolite for 2-EH and concluded it is not a reproductive toxicant based on the results from a GLP-compliant oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test conducted according to OECD Guideline 422 and an extended one generation reproductive toxicity study (EOGRTS) conducted according to OECD Guideline 443 and GLP.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *L*
2-EHA was assigned a score of Low for developmental toxicity based on the lack of developmental effects in developmental toxicity studies conducted with its hydrolysis products, acrylic acid and 2-ethyhexanol. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate negative data are available, and they are not GHS classified (CPA 2018b).
Although the score is based on measured data of high quality for strong surrogates and a conclusion from an authoritative body for the surrogate 2-ethyhexanol, the confidence in the score is reduced due to

an OECD Guideline 414 (oral Prenatal Developmental Toxicity test) study on the target chemical being underway, and the results will take precedence over surrogate data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
    - *Oral*: In the previously described GLP-compliant repeated dose toxicity study with a reproductive and developmental screening toxicity study conducted according to OECD Guideline 422, Sprague-Dawley rats, (10/sex/dose) were administered 2-EHA (99.518% purity) via gavage at doses of 0, 75, 250, 750 or 1,000 mg/kg/day. Males were treated for a total of 28 days beginning 14 days prior to mating and through sacrifice on day 28. Females were exposed for 41-55 days, i.e., during 2 weeks prior to mating, during mating, during post-coitum, and until day 13 of lactation. The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, clinical chemistry, reproductive performance (male or female mating index, male or female fertility index, male copulation index, number of corpora lutea, number of implantations), gross pathology, and histopathology. Offspring were evaluated for survival, mean litter size, sex ratio, body weight, and external and internal abnormalities. Treatment caused effects on postnatal survival, and mean pup body weights in the 750 and 1,000 mg/kg/day groups. Based on this authors assigned a NOEL of 250 mg/kg/day for F1 neonatal toxicity. Authors of REACH dossier, however, stated that the findings from this study are not clear analytical verification of the dose formulations showed large variabilities (33 - 131 %) and several dosing formulations did not meet the acceptability criteria. Therefore, the study was considered not reliable (Klimisch 4, not assignable).
    - *Inhalation***:** In a developmental toxicity study, pregnant Sprague-Dawley rats (20/dose) were exposed to 2-EHA (99.7% purity) vapor via whole body inhalation at concentrations of 50, 75, and 100 ppm (corresponding to approx. 0.38, 0.56, and 0.75 mg/L according to ECHA record) for 6 hours per day on days 6 through 20 of gestation. Maternal examinations included body weight, food consumption, and ovarian and uterine content. Fetal examinations included number of live and dead fetuses, fetal weight, fetal sex, and the incidence of external, visceral, and skeletal malformations. No embryo-/fetotoxic effects were revealed even at the highest tested concentration at which some signs of maternal toxicity had been observed. Therefore, a NOAEC of 100 ppm (approximately 0.750 mg/l) was established for developmental toxicity. Based on slightly reduced food intake and lower maternal weight gain at the higher exposure level a NOAEC of 75 ppm (approximately 0.563 mg/L) was established for maternal toxicity. Due to technical limitations exposure to higher concentrations could not be tested (Klimisch 2, reliable with restrictions).
- ECHA 2021b,c
    - *Oral*: *Surrogate: Acrylic acid (CAS #79-10-7):* In the previously described one-generation reproductive toxicity study, males and 20 females per group) received doses of 0, 83, 250, or 750 mg/kg/day for 13 weeks. Pups in the 750 mg/kg/day group had decreased body weight gain, a reduction in absolute and absolute liver weights in males, and a reduction in both absolute and relative spleen weights in females, suggesting postnatal developmental toxicity at a maternally toxic dose (Klimisch 2, reliable with restrictions).
    - *Oral*: *Surrogate: Acrylic acid (CAS #79-10-7):* In the previously described two-generation toxicity study, Wistar rats received doses of 0, 53, 249, or 460 mg/kg/day in their drinking water. There was a dose-dependent decrease in food and water consumption and a concomitant decrease in body weight and weight gain in both sexes in the F_1 generation at

240 mg/kg/day group. The authors identified a NOAEL of 53 mg/kg/day (Klimisch 1, reliable without restriction).

- *Inhalation*: Surrogate: Acrylic acid (CAS #79-10-7): In a developmental toxicity study, pregnant Sprague-Dawley rats (30/group) were exposed to 0, 120, 350, or 1,060 mg/m^3 (equivalent to 0, 0.120, 0.350, and 1.060 mg/L) via whole body inhalation for 6 hours per day during gestation days 6 -15. Dams were observed until day 20 of gestation. Treatment with 0.350 and 1.060 mg/L produced a dose-dependent decrease in food and water intake resulting in decreased body weight. There were no changes in pre-implantation losses, live fetuses, or resorptions. Further, treatment did not alter the incidences of abnormalities, variations, or retardations in fetuses in terms of general appearance, fetal body weight, or the condition of internal organs or the skeleton (Klimisch 1, reliable without restriction).
- *Inhalation*: Surrogate: Acrylic acid (CAS #79-10-7): In a developmental toxicity study, pregnant New Zealand rabbits (16/group) were exposed to 1, 25, 75, or 225 ppm (equivalent to 0.074, 0.22, 0.66 mg/L[9]) via whole-body inhalation for 6 hours per day during days 6 – 18 of gestation. Treatment had no effect on the number of ovarian corpora lutea, and the number of total viable or non-viable implantations/litter. The percentage live fetuses and sex ratio were unaffected. Treatment did not alter fetal body weight, or cause skeletal malformations or variations (Klimisch 1, reliable without restriction).

- ECHA 2015, 2021b,
  - Surrogate: 2-EH (CAS #104-76-7): ECHA evaluated the developmental toxicity of 2-EH and concluded it is not a developmental toxicant based on the absence of developmental effects in mice and rats at doses not lethal to mothers, in studies performed in compliance with OECD Guidelines. Only at high doses, which were lethal to dams, 2-EH increases intrauterine lethality of embryos and pups and leads to retardation of development. Even at these lethal doses the increase of fetal malformations is very small, and no dose-response relationship is seen. Therefore, ECHA concluded that these developmental effects at doses highly toxic to dams are secondary non-specific consequence of other toxic effects in dams, and they do not justify classification of 2-EH for developmental toxicity per GHS. This is supported by data on 2-ethylhexanoic acid, the major metabolite of 2-EH, which ECHA concluded to not be a developmental toxicant (ECHA 2017a). Study summaries for 2-EH are provided below.
- ECHA 2021d
  - *Oral*: Surrogate: 2-EH (CAS #104-76-7): A GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414 was performed with pregnant female CD-1 mice (28/dose group) provided diets containing 2-EH (greater than 99% purity) at 0%, 0.009%, 0.03%, or 0.09% (equivalent to 0, 17, 59, and 191 mg/kg/day, respectively) on GD 0-17. The animals were sacrificed on GD 17. Maternal examinations included body weight, food consumption, and ovarian and uterine content. Fetal examinations included number of live and dead fetuses, fetal weight, fetal sex, and the incidence of external, visceral, and skeletal malformations. No treatment-related maternal toxicity was observed. No treatment-related effects were observed on the number of corpora lutea, uterine implantation sites (live, dead, resorbed), pre- and post-implantation loss, sex ratio, or live fetal body weight per litter (all fetuses or separately by sex). Treatment with 2-EH did not increase the incidence of individual or total external, visceral, or skeletal malformations or variations. The study

[9] Conversion from ppm to mg/L (assuming normal temperature and pressure):
$\frac{(25\ ppm)(72.0626)}{24{,}450} = 0.074\ mg/L$

authors identified a maternal toxicity and developmental toxicity NOAEL of 191 mg/kg/day (Klimisch 1, reliable without restriction).

- *Oral*: *Surrogate: 2-EH (CAS #104-76-7):* A GLP-compliant developmental toxicity study conducted according to EU Method B.31/OECD Guideline 414 was performed with female Wistar rats (10/group). Rats were administered 2-EH at doses of 0, 130, 650, and 1,300 mg/kg/day via gavage on GD 6 through 15. At 1,300 mg/kg/day significantly reduced food consumption was measured in all parental dose groups. Severe clinical symptoms were observed including abdominal or lateral position, unsteady gait and apathy. Discoloration of the liver, lung edema, and emphysema were also reported in parental animals of the top dose group. An increased number of resorptions and markedly increased post implantation loss, along with increased resorptions, decreased fetal body weights and increased incidence of fetuses with dilated renal pelvis and/or skeletal malformations were observed. At 650 mg/kg/day the only reported maternal effects were two dams with piloerection. Pups of dams dosed with 650 mg/kg/day displayed a reduction in mean fetal body weights and increased frequency of fetuses with skeletal variations and retardations. At 130 mg/kg/day no substance related effects were reported. Based on available data, ToxServices established a NOAEL and LOAEL of 130 and 650 mg/kg/day due to reduced fetal body weights and increased skeletal variations in pups.
  - *This study was assigned a reliability score of 4 (not assignable) in ECHA (2021d) due to various deficiencies in data analysis and deviations from the current OECD Guideline.*
- *Dermal*: *Surrogate: 2-EH (CAS #104-76-7):* A GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414 was performed with pregnant female F344 rats (25/group) administered dermal doses of undiluted 2-EH (> 99.7% purity) at 0, 252, 840, or 2,520 mg/kg/day via occluded cutaneous application on GD 6 to 15. Maternal evaluations included body weight, food consumption, and ovarian and uterine content. Fetal examinations included assessment of external, visceral, skeletal, and head malformations and variations. Maternal toxicity was observed in the high dose group as decreased body weight gain. No treatment-related increases were measured in the incidence of fetal malformations. The study authors identified a maternal toxicity NOAEL of 840 mg/kg/day and a developmental toxicity NOAEL of 2,250 mg/kg/day (Klimisch 1, reliable without restriction).
- *Inhalation*: *Surrogate: 2-EH (CAS #104-76-7):* A developmental toxicity study (GLP status not reported) conducted in a manner similar to OECD Guideline 414 was performed with pregnant female Sprague-Dawley rats (15/concentration group) exposed to whole body exposures to 2-EH (greater than 99% purity) at 0 or 0.85 mg/L 7 hours/day on gestation days (GD) 1 to 19. 2-EH reduced maternal feed intake. No fetal toxicity or increased malformations were reported. A NOAEL of 0.850 mg/L was established by the study authors. No further details were available (Klimisch 2, reliable with restrictions).

Endocrine Activity (E) Score (H, M, or L): DG

2-EHA was assigned a score of Data Gap for endocrine activity due to lack of sufficient data. Although *in vitro* high throughput and *in silico* modeling do not indicate a concern for endocrine effects, no *in vivo* data are available.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2021b

  - 2-EHA was active in 1/18 estrogen receptor (ER) assays, 0/14 androgen receptor (AR) assays, 0/2 steroidogenesis assays, and 4/15 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century (Appendix D).
  - 2-EHA was predicted to be inactive for estrogen agonism, antagonism and binding according to the CERAPP Potency Level models (Consensus and from literature). It was also predicted to be inactive for androgen receptor agonism, antagonism and binding according to the COMPARA (Consensus) model (Appendix E).
  - *Surrogate: 2-EH (CAS #104-76-7):* 2-EH was inactive for estrogen receptor agonism and antagonism in 18 out of 18 assays, inactive for androgen receptor agonism or antagonism in 14 out of 14 assays, inactive for thyroid receptor activity in 10 out of 10 assays, and inactive for steroidogenesis receptor in 2 out of 2 assays of the Tox 21 high throughput *in vitro* assays (Appendix F).
- TEDX 2017
  - *Surrogate: 2-EH (CAS #104-76-7):* 2-EH is classified as a Potential Endocrine Disruptor by the TEDX screening list based on the following studies:
    - Male C57BL/6J mice were administered prenatal exposures to a mixture of 23 oil and gas operation chemicals (presumable including 2-EH but this could not be verified via the study abstract) at 3, 30, or 300 µg/kg/day and assessed for reproductive and developmental outcomes. Prenatal exposure to this mixture resulted in decreased sperm counts, increased body, testes, heart, and thymus weights, and increased serum testosterone levels (Kassotis et al. 2015).
    - Human endometrial cancer cells with a reporter gene were exposed to 24 chemicals used (presumable including 2-EH but this could not be verified via the study abstract) and/or produced by oil and gas operations. Twenty-three of these chemicals activated or inhibited the estrogen, androgen, glucocorticoid, progesterone, and/or thyroid receptors. Furthermore, mixtures of these chemicals acted synergistically, additively, or antagonistically in this cell system (Kassotis et al. 2015).
    - Water samples collected from a hydraulic fracturing-dense region were solid-phase extracted and measured for estrogen and androgen receptor activities using human cell lines with reporter gene assays. Estrogenic, anti-estrogenic, androgenic, and anti-androgenic activities were detected in 89%, 41%, 12%, and 46% of 39 unique water samples, respectively. Evaluation of a subset of natural gas drilling chemicals (presumable including 2-EH but this could not be verified via the study abstract) revealed novel anti-estrogenic, novel anti-androgenic, and limited estrogenic activities (Kassotis et al. 2014).
- Based on the weight of evidence, a score of DG was assigned. According to GreenScreen® criteria, listing in TEDX corresponds to a score of Moderate or High. TEDX broadly considers effects to be evidence of endocrine disruption when they are related to the "reproductive system, fetal development, the nervous system and behavior, the immune and metabolic systems, gene expression, and many other organs, glands and tissues"[10]. The basis of the surrogate 2-EH's classification is not clear as the above three studies that TEDX referred to as the evidence for the classification were conducted on mixtures containing 2-EH and effects could be probably attributed to chemicals present in the mixture other than 2-EH. In addition, effects reported in these studies (decrease in sperm counts, increased body, testes, heart, and thymus weights, and estrogenic, anti-estrogenic, androgenic, and anti-androgenic activities) were not seen in the relevant *in vivo* or in

[10] https://www.endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/methodology

vitro studies available for 2-EH. Further, the *in vitro* high throughput and *in silico* modeling for 2-EHA and its metabolite 2-EH do not indicate a concern for endocrine effects. Accordingly, ToxServices disregarded the TEDX listing for the metabolite 2-EH and assigned a score of DG for this endpoint due to lack of sufficient *in vivo* data.

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II* endpoints are distinguished in the v 1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen® Guidance v1.4, Annex 2 for more details.

Acute Mammalian Toxicity (AT) (Group II) Score (vH, H, M, or L): L

2-EHA was assigned a score of Low for acute toxicity based on oral and dermal LD_{50} values greater than 2,000 mg/kg and an inhalation LC_0 of greater than its saturated vapor concentration. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral and dermal LD_{50} values are greater than 2,000 mg/kg, inhalation LC_{50} values are 20 mg/L (vapor), and when they are not GHS classified or classified to GHS Category 5 (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS - New Zealand - 6.1E (oral) - Acutely toxic (GHS Category 5)
- ECHA 2021b (Note: Studies reported in the REACH dossier with reliability scores of 3 (not reliable) or 4 (not assignable) were not included as the available studies with higher reliability scores were sufficient to assess this endpoint)
  - *Oral*: LD_{50} (rat) = 4,435 mg/kg (similar to OECD Guideline 401) (Klimisch 2, reliable with restrictions).
  - *Oral*: LD_{50} (rat) = 5,766 mg/kg (Klimisch 2, reliable with restrictions).
  - *Oral*: LD_{50} (male mice) > 5,000 mg/kg (Klimisch 2, reliable with restrictions).
  - *Dermal*: LD_{50} (rabbit) ≥ 7,522 mg/kg from two studies (Klimisch 2, reliable with restrictions).
  - *Inhalation*: 8-hour whole body LC_0 (male and female rat) > 1.19 mg/L (saturated vapor concentration)

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score (vH, H, M, or L): M

2-EHA was assigned a score of Moderate for systemic toxicity (single dose) based on association with the authoritative list of EU-GHS (H335) which corresponds to GHS Category 3 (respiratory tract irritation). Although one of the acute inhalation toxicity studies did not show signs of respiratory irritation, the other study reported nasal irritation upon gross necropsy, and local irritant effects in the respiratory tract of rats were observed when they were exposed to low doses of the chemical during a repeated dose inhalation toxicity study (see Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) section below). GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when they are listed on the authoritative list of EU-GHS (H335) and classified as GHS Category 3 specific target organs/systemic toxicity following single exposure (CPA 2018b). The confidence in the score is high as it is based on an authoritative list.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) - H335 - May cause respiratory irritation [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]

  - *Screening:* GHS – Australia - H335 - May cause respiratory irritation [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]
- ECHA 2021b
  - *Oral:* In the acute oral toxicity study similar to OECD Guideline 401, rats (sex and strain not specified) were given 10% aqueous solution of 2-EHA (stabilized with 0.05% hydroquinone, no data on purity) at single doses of approximately 2, 3, and 5 mL/kg (corresponding to 1,809.5, 2,802.9, and 4,443.9 mg/kg as calculated by the study authors). An observation period of 7 days followed. Treated animals at the high dose showed clinical signs of apathy, narcotic state, and diarrhea. Necropsy of surviving rats did not find any abnormality. Authors identified an oral LD_{50} of approximately of 5.0 ml/kg (approximately 4,430 mg/kg) (Klimisch 2, reliable with restrictions).
  - *Oral:* In an acute oral toxicity test, CD-1 male mice (10/dose group) were administered 2-EHA (purity > 99.5%, stabilized with 10-20 ppm MMHQ) in corn oil via gavage at single doses of 2,500 and 5,000 mg/kg. An observation period of 14 days followed. No mortality was seen after administration of 2,500 mg/kg, but 2/10 mice died within 24 hours after administration of 5,000 mg/kg. Surviving animals recovered within 3 days after substance application. Clinical signs observed were scant droppings, wet yellow stained anogenital area, decreased spontaneous motor activity, ataxia, and abdominal breathing. No gross changes were detected at necropsy. Authors identified an oral LD_{50} of > 5,000 mg/kg (Klimisch 2, reliable with restrictions).
  - *Dermal*: In the acute dermal toxicity studies that reported dermal LD_{50} values of ≥ 7,522 mg/kg, no details on clinical signs of toxicity or body weight or histopathological examination were reported.
  - *Inhalation*: In an acute inhalation toxicity study similar to OECD Guideline 403, male and female rats (3/sex/dose, strain not specified) were exposed to an atmosphere saturated with 2-EHA (stabilized with 0.05% hydroquinone, no data on purity) vapor via whole body exposure for 8 hours. No analytical determination of the atmosphere concentrations was performed. The vapor saturation (1.19 mg/L) was calculated based on the vapor pressure at 25°C and the molecular weight. Animals were observed for 14 days following the exposure. No mortality and no clinical signs were observed and no necropsy examination was performed (Klimisch 2, reliable with restrictions).
  - *Inhalation*: In a range-finding test on 2-EHA, male and female albino rats (3/sex/dose) were exposed to atmosphere saturated with 2-EHA (no data on purity) vapor via whole body exposure for 8 hours. Hyperactivity on removal from exposure chamber was the only clinical sign documented, gross pathology revealed nasal and ocular irritation (Klimisch 2, reliable with restrictions).

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*) Score (H, M, or L): *M*

2-EHA was assigned a score of Moderate for systemic toxicity (repeated dose) based on an inhalation LOAEC of 0.566 mg/L/6h/day established in a 90-day study in rats classifying it to GHS Category 2. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when animal studies identify inhalation LOAEC values between 0.2 and 1.0 mg/L/6h/day in 90-day studies and when they are classified to GHS Category 2 (CPA 2018b). The confidence in the score is low as there is insufficient information to conclude that adverse effects do not occur at 0.2 mg/L/6h/day.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.

- ECHA 2021b
    - *Oral*: In the previously described GLP-compliant repeated dose toxicity study with a reproductive and developmental toxicity screening study conducted according to OECD Guideline 422, Sprague-Dawley rats, (10/sex/dose) were administered 2-EHA (99.518% purity) via gavage at doses of 0, 75, 250, 750 or 1,000 mg/kg/day. Males were treated for a total of 28 days beginning 14 days prior to mating and through sacrifice on day 28. Females were exposed for 41-55 days, i.e., during 2 weeks prior to mating, during mating, during post-coitum, and until day 13 of lactation. The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, clinical chemistry, reproductive performance (male or female mating index, male or female fertility index, male copulation index, number of corpora lutea, number of implantations), gross pathology, and histopathology. Offspring were evaluated for survival, mean litter size, sex ratio, body weight, and external and internal abnormalities. Treatment caused parental systemic effects in males at doses from 250 mg/kg/day and females at 750 mg/kg/day as characterized by clinical observations and microscopic changes in the stomach and liver. Therefore, authors assigned systemic toxicity NOAELs of 75 mg/kg/day for males and 250 mg/kg/day for females. *The LOAEL of 250 mg/kg/day is above the duration-adjusted GHS guideline value for Category 2 of 200 mg/kg/day for a ~45 day oral study[11]. Therefore, 2-EHA is not classified per GHS. However, ToxServices did not use this study for the assessment of this endpoint due to the study being considered not reliable by the authors of REACH dossier (Klimisch 4, not assignable) as stated previously.*
    - *Inhalation*: In a GLP-compliant subchronic repeated inhalation exposure toxicity study conducted according to OECD Guideline 413, Wistar rats (10/sex/concentration group) were administered whole body exposures to 2-EHA (99.7% purity) vapor at 0, 10, 30 or 100 ppm (approximately 0.075 mg/L, 0.226 mg/L or 0.753 mg/L for the treatment groups as calculated by the study authors) for 6 hours/day, 5 days/week for 90 days (equivalent to a total of 65 exposures). The animals were evaluated for clinical signs of toxicity, body weight, body weight gain, hematology, clinical chemistry, gross pathology, and histopathology. No mortalities occurred. Animals at the high and mid doses exhibited lethargy and ptosis. Further, treatment caused a decrease of the body weight gain and clinical chemistry changes (elevated activities of transaminase and alkaline phosphatase) in animals at the high dose. The microscopic examination revealed no lesion other than a focal or diffuse degeneration of the olfactory epithelium of the cranial nasal cavity in animals of both sexes of the high and mid dose groups. All rats of the 100 ppm group showed degeneration of the olfactory mucosa in the anterior part of the nasal cavity. The incidence of degeneration of the olfactory mucosa but not the severity was increased in mid dose rats. No treatment-related lesion of the nasal cavity was diagnosed at the low dose level. Degeneration of the olfactory epithelium was characterized by a reduction of cell layers, reduction or loss of apical cytoplasmic structures such as olfactory knobs and microvilli. Based on this, the study authors identified a NOAEC of 0.075 mg/L (10 ppm) for local effects (degeneration of the olfactory epithelial layer in the cranial part of the nasal cavity). The NOAEC for systemic effects was 0.226 mg/L (30 ppm) based on decreased body weight gain (Klimisch 2, reliable with restrictions). *The LOAEC of 0.753 mg/L (100 ppm), which is equivalent to 0.538 mg/L/6h/day[12], is within the GHS Category 2 Guidance values of 0.2 -1 mg/L/6h/day for vapors in a 90-day study. Therefore 2-EHA is classified to GHS Category 2 for systemic toxicity following repeated exposure. The NOAEC of 0.226 mg/L (equivalent to*

[11] 100 mg/kg/day x 90 days /45 days = 219.5 mg/kg/day
[12] Converting exposure period 5 days/week to daily = 0.753 mg/L x 5 / 7(days) = 0.538 mg/L/day

0.16 mg/L/6h/day) is below the threshold of 0.2 mg/L/6h/day. Therefore, there is insufficient information to conclude that adverse effects do not occur at 0.2 mg/L/6h/day.

- *Inhalation*: Additional inhalation repeated dose toxicity studies were identified in the REACH dossier; however, they were assigned reliability scores of 3 (not reliable) or involved treatment for only 9-11 days. Therefore, ToxServices did not include these studies in this GreenScreen® assessment.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *M*

2-EHA was assigned a score of Moderate for neurotoxicity (single dose) based on transient narcotic effects seen in acute oral toxicity studies classifying it to GHS Category 3. GreenScreen® criteria classify chemicals as a Moderate hazard for neurotoxicity (single dose) when they are classified to GHS Category 3 for narcotic effects (CPA 2018b). The confidence in the score is low as narcotic effects were observed in these studies at near fatal or fatal doses (> 2,000 mg/kg), which may suggest general toxicity rather than specific neurotoxicity.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
  - *Oral:* In the previously described key acute oral toxicity study similar to OECD Guideline 401, rats (sex and strain not specified) were given 10% aqueous solution of 2-EHA (stabilized with 0.05% hydroquinone, no data on purity) at single doses of approximately 2, 3, and 5 mL/kg (corresponding to 1,809.5, 2,802.9, and 4,443.9 mg/kg as calculated by the study authors). An observation period of 7 days followed. Treated animals at the high dose showed clinical signs of neurotoxicity such as apathy, and narcotic state. Necropsy of surviving rats did not find any abnormality. Authors identified an oral LD_{50} of approximately of 5.0 ml/kg (approximately 4,430 mg/kg) (Klimisch 2, reliable with restrictions).
  - *Oral:* In the previously described acute oral toxicity test, CD-1 male mice (10/dose group) were administered 2-EHA (purity > 99.5%, stabilized with 10-20 ppm MMHQ) in corn oil via gavage at single doses of 2,500 and 5,000 mg/kg. An observation period of 14 days followed. No mortality was seen after administration of 2,500 mg/kg, but 2/10 mice died within 24 hours after administration of 5,000 mg/kg. Surviving animals recovered within 3 days after substance application. Clinical signs of neurotoxicity were observed such as decreased spontaneous motor activity and ataxia. No gross changes were detected at necropsy. Authors identified an oral LD_{50} of > 5,000 mg/kg (Klimisch 2, reliable with restrictions).
- Based on a weight of evidence, a score of Moderate was assigned. The above data indicate acute exposure to the 2-EHA via the oral route caused reversible clinical signs of neurotoxicity such as narcotic state, decreased spontaneous motor activity, and ataxia. These observations are consistent with transient narcotic effects that warrant a GHS Category 3 classification, which corresponds to a score of Moderate.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): DG

2-EHA was assigned a score of Data Gap for neurotoxicity (repeated dose) based on a lack of data for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.

- No data were identified.

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): M

2-EHA was assigned a score of Moderate for skin sensitization based on evidence of skin sensitization in animals classifying it to GHS Category 1B and association with the authoritative list of EU-GHS H317 and MAK Sh. GreenScreen® criteria classify chemicals as a Moderate hazard for skin sensitization when they are classified to GHS Category 1B for skin sensitization (CPA 2018b). The confidence in the score is high as it is based on high quality experimental data and on authoritative lists.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) - H317 - May cause an allergic skin reaction [Skin sensitization - Category 1]
  - *Authoritative:* MAK - Sensitizing Substance Sh - Danger of skin sensitization
  - *Screening:* GHS - New Zealand - 6.5B (contact) - Contact sensitisers (Cat. 1*)*
  - *Screening:* GHS – Australia - H317 - May cause an allergic skin reaction [Skin sensitization - Category 1]
- ECHA 2021b
  - In a mouse local lymph node assay (LLNA) conducted according to OECD Guideline 429 and GLP, female CBA mice (5/dose group) were administered 2-EHA (purity not reported) at concentrations of 2%, 10%, 30% (v/v) in acetone: olive oil (AOO) and 100% for three consecutive days (days 1, 2 and 3) on the dorsum of both ears (25mL per ear). One group served as a vehicle control and was treated with AOO, and another group served as a positive control and was treated with a-hexylcinnamaldehyde (HCA) at a concentration of 25% (v/v) in AOO. Following the final application, the animals were sacrificed and the lymph nodes isolated to perform the proliferation assay. The mean stimulation indices (SI) for the 2%, 10%, 30% (v/v) and 100% were 1.19, 2.57, 3.53 and 5.50, respectively. As the SI value at 30 and 100%% were above 3, the estimated concentration (EC3) value giving rise to a 3 fold increase in lymphocyte proliferation was determined. The EC3 value obtained for 2-EHA was 18.96% (4,740 µg/cm²). Based on this, authors concluded that 2-EHA demonstrated a weak dermal sensitization potential in the local Lymph node assay (Klimisch 1, reliable without restriction). *As the EC3 value is > 2, 2-EHA is classified to GHS Category 1B for skin sensitization (UN 2019).*
  - In a second LLNA study conducted according to OECD Guideline 429 and GLP, female CBA mice (4/dose group) were administered 2-EHA (99.8% purity) at 0.5, 1, 2.5, 5 or 10% w/v in AOO (4:1). A vehicle control group was similarly treated using AOO alone. The mice were administered 25 µL of the test substance to the dorsal surface of each ear for 3 consecutive days. Following the final application, the animals were sacrificed and the lymph nodes isolated to perform the proliferation assay. The SI values for the 0.5, 1, 2.5, 5 and 10% doses were 1.1, 1.2, 1.0, 1.2 and 3.1, respectively. As the SI value at 10% was above 3, the EC3 was determined. The calculated EC3 was 9.7%w/v indicative of a sensitizer of moderate potency. Based on this, authors concluded that 2-EHA is a weak skin sensitizer and is classified to GHS Category 1B (Klimisch 2, reliable with restrictions).
  - In a third LLNA study, the potential of 2-EHA to induce a secondary immune response was examined using four test groups and three control groups of four female CBA mice each. The experiment was divided into two parts, induction (50%) and challenge phase (10%, 50%). In the induction phase, 5 female CBA/CaOlaHsd mice each were treated three times with a 50% (w/w) preparation of 2-EHA in AOO (4:1) or with the vehicle alone. During the challenge treatment, the animals were treated once with 1%, 2.5%, 10% and 50% (w/w) preparations of 2-EHA in AOO (4:1) or with the vehicle alone. It was concluded that 2-

EHA exhibits a skin sensitizing potential and is able to induce a secondary immune response. The threshold concentration for inducing a secondary immune response (elicitation threshold) of 2-EHA was > 2.5% (Klimisch 1, reliable without restriction).

- 2-EHA was also predicted to be a skin sensitizer in a battery of *in vitro* assays addressing key steps of the adverse outcome pathway (AOP) for skin sensitization as defined by OECD. The following tests have been conducted to assess the skin sensitizing potential of 2-EHA: protein reactivity (DPRA), activation of keratinocytes (LuSens), activation of dendritic cells (MUSST), and human Cell Line Activation Test (h-CLAT). A brief description of each assay is provided below:
  - In the DPRA assay, which was conducted in a manner similar to OECD Guideline 442C, the amount of proteins with nucleophilic side chains such as cysteine or lysine residues after incubation with putative allergens (2-EHA) was measured. For 2-EHA, the mean peptide depletion as average of cysteine- and lysine-peptide depletions was calculated to be 60.5% and thus, showing a high chemical reactivity (Klimisch 1, reliable without restriction).
  - In the LuSens assay, which was conducted in a manner similar to OECD Guideline 442D, the cell line Lu Sens was treated with 2-EHA at concentrations of 5.36 -27.67 µg/ml for 48 hours in at least two independent experiments with 3 replicates each. Cells were lysed and luciferase induction was evaluated by measuring luminescence signal after substrate addition. In parallel, a MTT assay was performed to assess cytotoxicity. A test substance was considered to have an antioxidant response element induction potential if the fold induction of luciferase activity was >1 .5 and viability determined in the MTT assay was >70% at any test concentration. Luciferase activity after 2-EHA treatment exceeded 1.5 fold induction with respect to the vehicle control at concentrations that did not reduce cell viability below 70% in two independent experiments (Klimisch 1, reliable without restriction).
  - The myeloid U937 skin sensitization test (OECD Guideline 442E) is a dendritic cell activation test (MUSST) to predict skin sensitizing potential. The test is performed using the human pro-monocytic cell line U937 as a surrogate for dendritic cells. As readout, the change in the expression of the cell membrane marker CD 86 measured by flow cytometry after 48 hours of test substance exposure is determined. A test substance is predicted to activate dendritic cells when CD86 cell surface expression exceeds the threshold of 1.2 in relation to vehicle control in at least two independent experiments. After 48 hours of exposure to 2-EHA, CD 86 expression was induced in U937 cells at concentration 125 µg/mL affording at least 70% viability. From this, authors concluded that 2-EHA did activate dendritic cells (Klimisch 1, reliable without restriction).
  - The human Cell Line Activation Test (h-CLAT) is an *in vitro* skin sensitization test based on the enhancement by sensitizers of CD86 and/or CD54 expression on THP-1 cells. After 24 hours of exposure to 2-EHA, CD86 expression was induced in THP-1 cells at concentrations between 279.1 and 1,000 µg/ml affording at least 50% viability. CD 54 expression was not induced in THP-1 cells at maximal test concentration and at any tested doses. From this, authors concluded that the test substance did activate dendritic cells (Klimisch 1, reliable without restriction).

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *M*

2-EHA was assigned a score of Moderate for respiratory sensitization based on being listed as Asthmagen by the CHE - Toxicant Database, a presence of a structural alert for respiratory sensitization and according to ECHA's recommended strategy on evaluation of respiratory sensitization.

GreenScreen® criteria classify chemicals as a Moderate hazard for respiratory sensitization when they are classified to GHS Category 1B (low to moderate frequency of occurrence) (CPA 2018b). The confidence in the score is low due to lack of experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists of for this endpoint.
  - *Screening:* Not listed on any screening lists of for this endpoint.
  - *Others:* CHE - Toxicant Database - Asthma - allergen, sensitizer - strong evidence
- OECD 2020a
  - The structure of 2-EHA was evaluated for alerts for respiratory sensitization using the OECD QSAR toolbox. One structural alert for respiratory sensitization was identified (Michael Addition) (See Appendix G).
- Based on the weight of evidence, a score of Moderate was assigned. 2-EHA is listed as an Asthmagen by the CHE - Toxicant Database and contains a structural alert for respiratory sensitization. In addition, it is a dermal sensitizer and according to ECHA's guideline (ECHA 2017b), a classification per GHS should be considered. Per ECHA's guideline (ECHA 2017b), the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017b). ECHA recommended that if a chemical is a dermal sensitizer based on high quality data, and contains a structural alert for respiratory sensitization, it should be classified as a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017b). 2-EHA contains a structural alert for respiratory sensitization, and is a skin sensitizer based on positive experimental data (see skin sensitization section above). Therefore, it is classifiable as a respiratory sensitizer. No information is available to subcategorize it to GHS Category 1A/1B. However, based on weak dermal sensitization potential and common mechanism of sensitization, ToxServices classified 2-EHA as a weak respiratory sensitizer (1B).

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): H

2-EHA was assigned a score of High for skin irritation/corrosivity based on association with the authoritative list of EU-GHS H315 which corresponds to GHS Category 2, supported by *in vitro/in vivo* data. GreenScreen® criteria classify chemicals as a High hazard for skin irritation/corrosivity when they are listed on the authoritative list of EU-GHS H315 and classified as GHS Category 2 dermal irritants (CPA 2018b). The confidence in the score is high as it is based on an authoritative list and measured data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) - H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]
  - *Screening:* GHS – Australia - H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]
  - *Screening*: GHS - New Zealand - 6.3A - Irritating to the skin (Cat. 2)
- ECHA 2021b
  - In a dermal irritation test conducted according to an internal method by BASF, an unspecified amount of 2-EHA (99% purity) was applied to the clipped skin of two Vienna White rabbits under occlusive condition for 1 min, 5 min, 15 min, and 20 hours. Treatment caused moderate erythema after1 and 5 minutes which reversed within 72 hours. After an exposure time of 15 minutes under occlusive conditions severe erythema with scaling after 8 days were observed and severe erythema (mean scores over 24/48/72 hours: 2.8) and moderate edema (mean scores over 24/48/72 hours: 1.3) appeared within 24 hours after a 20-

hour exposure time. A second trial was conducted with a 20-hour exposure under occlusion and direct comparison of 4 acrylates were made. Moderate-severe erythema and slight edema were observed with mean scores over 24/48/72 hours of 2.2 for erythema and 0.8 for edema. At the end of the observation period (8 days) desquamation was again reported. Based on the results from this study, authors classified 2-EHA to GHS Category 2 for skin irritation (Klimisch 2, reliable with restrictions).

- In an occlusive patch test conducted according to U.S. Federal Register Guideline of 1964, four New Zealand White rabbits were administered 0.5 mL of 2-EHA (purity not specified) to intact and abraded skin for 24 hours under occlusive condition. The mean scores (24 hours/72 hours) were 1.75/2 for erythema and 3.25/3.25 for edema for both intact and abraded skin. The primary irritation score was determined to be 5.0, indicating the potential of the substance to be a primary skin irritant. According to the Draize score, 2-EHA was found to be a moderate to severe primary irritant. Authors classified 2-EHA to GHS Category 2 for skin irritation (Klimisch 2, reliable with restrictions).
- 2-EHA (99.7% purity) was considered to be non-corrosive (GHS Category 1) to the skin when tested in a GLP-compliant *in vitro* skin irritation test conducted according to OECD Guideline 431 using reconstructed human Epidermis (RHE) Skin Model after treatment periods of 3 and 60 minutes and a 72-hour post-exposure incubation period. The relative mean viabilities of the test item were 99% after 3 minutes and 104% after 60 minutes, which were greater than 50% and indicated the substance was not irritating to the skin (Klimisch 1, reliable without restriction).
- Based on above data, authors of REACH dossier stated that 2-EHA should be classified as a skin irritant (GHS Category 2).

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): L

2-EHA was assigned a score of Low for eye irritation/corrosivity based on negative results in an ocular irritation test conducted according to OECD Guideline 405. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation when adequate data are available and negative, and they are not classified per GHS (CPA 2018b). Confidence in the score is high as it is based on experimental data of high quality for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS - New Zealand - 6.4A - Irritating to the eye (Cat. 2A)
    - Based on the results from the Carpenter study (NZ EPA 2021). *ToxServices evaluated the Carpenter study and considered it not appropriate for GHS classification purposes as the protocol applied was quite different from the OECD Guideline 405 study recommended by the GHS. In that study an observation period of 18-24-hour followed instead of 21 days as recommended in OECD guideline. In addition, the study used a scoring system significantly different from the Draize system according to OECD guidelines. Therefore, ToxServices disregarded the GHS-New Zealand listing. A brief description of the Carpenter study is provided here. An amount of 0.005 mL of the undiluted chemical was applied to the center of the cornea while the lids were retracted. About one minute later, the lids were released again. 18 to 24 hours later, the eye was examined in strong diffuse daylight, then stained with fluorescein, and the injury scored. Guided by the result, additional applications were made with varying dilutions and volumes until the chemical could be assigned to a grade. 2-EHA was graded 6/10 (0.005 mL yields*

score of 5.0, excess of 40% solution not over 5.0). Thus, the test substance was concluded to be a potential eye irritant.

- *Screening:* GHS – Japan - H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2]. *ToxServices notes that this classification was made in 2008, but was removed in 2020 (NITE 2020).*

- ECHA 2021b
  - In a GLP-compliant ocular irritation study conducted according to OECD Guideline 405, New Zealand rabbits (3 total) were administered ocular instillations of 0.1 mL undiluted 2-EHA (98% purity) for 24 hours. An observation period of 3 days followed the instillation. Treatment caused mild irritation with the mean scores at 24/48/72 hours of 0.3 for conjunctival redness and chemosis and 0 for corneal opacity and iritis. The conjunctival and chemosis effects were fully reversible within 3 days. Based on this, study authors concluded that 2-EHA was non-irritating to the eye (Klimisch 1, reliable without restriction).
  - In another ocular irritation study conducted according to BASF's internal study protocol, 2-EHA was not irritating to the eye when an amount of 0.5 mL undiluted was instilled into the eyes of Vienna White rabbits (n = 2) for 9 days. The eyes were not washed. After 1 hour erythema and edema were observed which had completely reversed within 48 hours in both animals. The mean scores at 24/48 hours were 0 for chemosis, cornea and iris and 0.5 for conjunctivae with effects being fully reversible within 48 hours (Klimisch 2, reliable with restrictions).
  - In an ocular irritation test conducted according to the U.S. Federal Register Guideline of 1964, 0.1 ml of undiluted 2-EHA (purity not specified) was instilled into the eyes of 6 rabbits for 72 hours. Treatment caused either slight or well-defined injection of the vessels of the conjunctivae. Scores after 24, 48, and 72 hours are stated to exist, but no information on these scores was available. The observation period was not mentioned. No corneal or iris lesions were identified in any animal. Authors of REACH dossier stated that since only 1/6 rabbits displayed a reaction which would be considered to be positive according to U.S. regulations of 1964, the test was regarded as being negative (Klimisch 2, reliable with restrictions).

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): H

2-EHA was assigned a score of High for acute aquatic toxicity based on acute aquatic toxicity values of 1.81, 1.3 and 1.71 mg/L in fish, *Daphnia* and algae, respectively. GreenScreen® criteria classify chemicals as a High hazard for acute aquatic toxicity when L/EC_{50} values are greater than 1 to 10 mg/L (CPA 2018b). The confidence in the score is high as it is based on measured data for all three trophic levels.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
  - 96-hour mortality LC_{50} (*Oncorhynchus mykiss*, fish) = 1.81 mg/L (99.7% purity, GLP-compliant, OECD Guideline 203) (Klimisch 1, reliable without restriction).
  - 48-hour mobility EC_{50} (*Daphnia magna,* invertebrate) = 1.3 mg/L (99.7% purity, GLP-compliant, OECD Guideline 202) (Klimisch 1, reliable without restriction).
  - 72-hour growth rate EC_{50} (*Desmodesmus subspicatus,* algae) = 1.71 mg/L (99.7% purity, GLP-compliant, OECD Guideline 201) (Klimisch 1, reliable without restriction).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *H*

2-EHA was assigned a score of High for chronic aquatic toxicity based on based on the 72-hour NOEC of 0.45 mg/L in algae and the 21-day EC_{10} values of 0.85 -0.91 mg/L in *Daphnia*. GreenScreen® criteria classify chemicals as a High hazard for chronic aquatic toxicity when chronic aquatic values are greater than 0.1 to 1 mg/L (CPA 2018b). The confidence in the score is low as no data were identified for the aquatic vertebrate trophic level for the target chemical or its surrogates.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
  - 21-day EC_{10} (*D. magna,* invertebrate) is 0.91 mg/L for reproduction and 0.85 mg/L for growth rate (GLP-compliant, OECD Guideline 211) (Klimisch 1, reliable without restriction).
  - 72-hour growth rate NOEC (*D. subspicatus,* algae) = 0.45 mg/L (99.7% purity, GLP-compliant, OECD Guideline 201) (Klimisch 1, reliable without restriction).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): L

2-EHA was assigned a score of Low for persistence based on meeting the GHS rapid degradation criteria when tested according to EU Method C.4-D (Manometric Respirometry Test) and on being predicted to predominantly partition to soil. GreenScreen® criteria classify chemicals as a Low hazard for persistence when they meet the rapid degradation criteria under GHS, and they primarily partition to soil, water or sediment (CPA 2018b). Confidence in the score is high as it is based on measured data of high quality for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b (Note: Studies reported in the REACH dossier with reliability scores of 3 (not reliable) or 4 (not assignable) were not included as the available studies with higher reliability scores were sufficient to assess the persistence endpoint)
  - In a ready biodegradability test (non-GLP) conducted according to EU Method C.4-D (Manometric Respirometry Test), domestic, non-adapted activated sludge was exposed to 2-EHA (purity not specified) at 100 mg/L for 28 days. A degradation rate of 70-80% was achieved within 15 days. The study authors concluded that 2-EHA was readily biodegradable in this study. No information was provided for the 10-day window (Klimisch 2, reliable with restrictions).
  - In a ready biodegradability test conducted according to OECD Guideline 301C (Modified MITI Test (I)), non-adapted activated sludge was exposed to 2-EHA (purity not specified) at a concentration of 100 mg/L for 14 days. A degradation rate of 50-60% was achieved after 14 days. Authors concluded that the test substance is moderately biodegradable under the test conditions (Klimisch 2, reliable with restrictions).
- U.S. EPA 2017
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that 2-EHA is expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 76%.8% will partition to soil with a half-life of 30 days (720 hours / 24 hours), 20.8% will partition to water with a half-life of 15 days, and 2.04% will partition to air with a half-life of 11.8 hours (Appendix H).

- Based on the weight of evidence, a score of Low was assigned. 2-EHA was readily biodegradable when tested according to EU Method C.4-D with a biodegradation rate of 70-80%. Although no information was provided on the 10-day window to assign a Very Low score, the available information indicates that it meets the GHS criteria for "rapid degradability" (reaching > 70% degradation in 28 days), which corresponds to a GreenScreen® score of Low. Modeling predicts that this chemical will partition primarily to soil.

Bioaccumulation (B) Score (vH, H, M, L, or vL): L
2-EHA was assigned a score of Low for bioaccumulation based on a measured BCF of 347. GreenScreen® criteria classify chemicals as a Low hazard for bioaccumulation when BCF values are > 100 to 500 (CPA 2018b). The confidence in the score is high as it is based on an experimental BCF value for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021b
  - 2-EHA has a measured log K_{ow} value of 4.26 at 25ºC obtained from a test similar to OECD Guideline 107 (Shake Flask Method) (Klimisch 2, reliable with restrictions).
  - In a GLP-compliant bioaccumulation test conducted according to OECD Guideline 305 (Aqueous and Dietary Exposure) using tissues of *Cyprinus carpio*, the kinetic bioconcentration factor (BCFk) for 2-EHA was determined based on total radioactivity which includes the sum of the parent compound, possible metabolites and assimilated carbon. The analysis of the extracts of the fish tissues showed no parent 2-EHA and based on total radioactivity the BCFk was 347 L/kg and the half-life (DT50) value of the total radioactivity was 19 days indicating slow depuration (Klimisch 1, reliable without restriction).
- Although the measured log K_{ow} value corresponds to a Moderate score, ToxServices relied on the measured BCF for this endpoint, as this value is the more reliable measure of true bioaccumulation potential compared to log K_{ow}, which is just a physicochemical property of the chemical.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
2-EHA was assigned a score of Low for reactivity based on the NFPA rating of 1 for physical hazard /reactivity supported by lack of structural alerts for oxidizing properties. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when available data indicate that the chemical does not warrant GHS classification for any of the reactivity sub-endpoints and the chemical is not present on authoritative or screening lists (CPA 2018b). The confidence in the score is low due to lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- No measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of 2-EHA. These procedures are listed in the GHS (UN 2019).
  - Based on its structure, 2-EHA contains a structural alert for explosivity, C-C unsaturation. However, it is not considered explosive or self-reactive in its REACH dossier (See Appendix I, ECHA 2021b).

  - Based on its structure, 2-EHA is not considered to have oxidizing properties as it does not contain any structural groups known to be correlated with a tendency to react exothermally with combustible materials.
- CAMEO 2021
  - 2-EHA it is reported to have a physical/reactivity hazard score of 1 from the NFPA which corresponds to "Materials that are normally stable but can become unstable at elevated temperatures and pressures.." [13].

Flammability (F) Score (vH, H, M, or L): M

2-EHA was assigned a score of Moderate for flammability based on ToxServices classifying it as a GHS Category 4 flammable liquid. GreenScreen® criteria classify chemicals as a Moderate hazard for flammability when they are classified as GHS Category 4 flammable chemicals (CPA 2018). The confidence in the score is high as it is based on a measured flash point for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Québec CSST - WHMIS 1998 - Class B3 - Combustible liquids
  - *Screening:* New Zealand - GHS - 3.1D - Flammable Liquids: low hazard
    - Based on a flash point of 86°C in a closed cup test (NZ EPA 2021).
- ECHA 2021b
  - In a closed cup test conducted according to ISO Guideline, 2-EHA had a flash point of 80°C at 101.3 kPa.
  - 2-EHA is classified as a GHS flammable liquid Category 4 (combustible liquid), because the flash point is >60°C and < 93° C.
- Based on the above information, ToxServices classified 2-EHA as a Category 4 flammable liquid under GHS criteria (UN 2019). GHS criteria define Category 4 flammable liquids as chemicals with flash points greater than 60°C to no greater than 93°C.

[13] https://www.fm.colostate.edu/files/forms/safety/CH-23.NFPA.ratings.pdf

Use of New Approach Methodologies (NAMs)[14] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity, endocrine activity, skin irritation, and skin sensitization and *in silico* models for respiratory sensitization and endocrine activity. NAMs are non-animal alternative that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020, OECD 2020b). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020b):

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

As shown in Table 4, Type I (input data) uncertainties in EHA's NAMs dataset include the absence of experimental data and established test methods for endocrine activity and respiratory sensitization. 2-EHA's Type II (extrapolation output) uncertainties include the limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, the limitation of the *in vitro* skin corrosion test **(**OECD Guidelines 431) to identify substances classified as skin irritants (GHS Category 2), the limitation of the *in vitro* skin sensitization assays to address chemicals that are pre-haptens, the unknown *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* modeling of receptor binding, and the lack of defined applicability domains in OECD Toolbox as well as ToxCast models. Some of the type I and type II errors can be alleviated by the use of genotoxicity test batteries, *in vivo* data for skin irritation and sensitization and ECHA's decision framework and guidance to evaluate respiratory sensitization.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020b)	
Type I Uncertainty: Data/Model Input	**Genotoxicity, skin sensitization and skin irritation:** No Type I uncertainty is identified on using the *in vitro* assays for genotoxicity, skin irritation and skin sensitization as they are considered relevant (appropriate for the evaluation of the corresponding hazards as recommended in the OECD Guideline), reliable (they have Klimisch scoring of 2 or 1) and adequate (validated methods). **Respiratory sensitization**: No experimental data or human data are available. In addition, there are no formally recognized and validated animal or *in vitro* tests. **Endocrine activity:** No *in vivo* experimental data or human data are available.
Type II Uncertainty: Extrapolation Output	**Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in

[14] NAMs refers to any non-animal technology, methodology, approach, or combination thereof that inform chemical hazard and risk assessments. NAMs include *in silico*/computational tools, *in vitro* biological profiling (e.g., cell cultures, 2,3-D organotypic culture systems, genomics/transcriptomics, organs on a chip), and frameworks (i.e., adverse outcome pathways (AOPs), defined approaches (DA), integrated approaches to testing and assessment (IATA).

	non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[15]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[16] **Endocrine activity:** ToxCast models don't define applicability domain; the *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* modeling of receptor binding is unknown due to lack of consideration of metabolism and other toxicokinetic factors. **Skin sensitization:** The *in silico* and *in vitro* assays evaluating key events in the skin sensitization AOP don't typically include metabolism or abiotic transformation to address chemicals that are pro-haptens or pre-haptens, respectively.[17] **Skin irritation**: The OECD 431 test is only used to identify corrosive substances (GHS Category 1)[18] It cannot identify skin irritant (Category 2) or mild skin irritant (GHS Category 3) (ECHA 2017). **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017b), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* micronucleus test
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays *In silico* modeling: ToxCast models
Acute mammalian toxicity	N	
Single exposure systemic	N	

[15] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[16] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[17] https://www.oecd-ilibrary.org/environment/test-no-442c-in-chemico-skin-sensitisation_9789264229709-en; https://www.oecd-ilibrary.org/environment/test-no-442d-in-vitro-skin-sensitisation_9789264229822-en; https://www.oecd-ilibrary.org/environment/test-no-442e-in-vitro-skin-sensitisation_9789264264359-en
[18] https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1614097188&id=id&accname=guest&checksum=5C0F2BF5F910961BDDD2D30A71941A7D

toxicity		
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	Y	*In vitro* test*:* OECD Guideline 442C (DPRA), OECD Guideline 442D (LuSens), OECD Guideline 442E (MUSST and h-CLAT).
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	Y	*In vitro* test: OECD Guideline 431 *in vitro* skin corrosion tests with reconstructed human epidermis (RHE) test method
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	N	
Bioaccumulation	N	

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APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for 2-EHA (CAS #103-11-7)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicit	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	**Chemical Name**	**CAS#**	**C**	**M**	**R**	**D**	**E**	**AT**	**STs**	**STr**	**Ns**	**Nr**	**SNS***	**SNR***	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
No	2-EHA	103-11-7	M	L	*L*	*L*	DG	L	M	*M*	*M*	DG	M	*M*	H	L	H	*H*	L	L	*L*	M

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	g
1	No	No	No	No	No		
2	No	No	No	No	Yes	No	No
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
2-EHA	2

Note: Chemical has not undergone a data gap assessment. Not a Final GreenScreen™ Score

Table 6

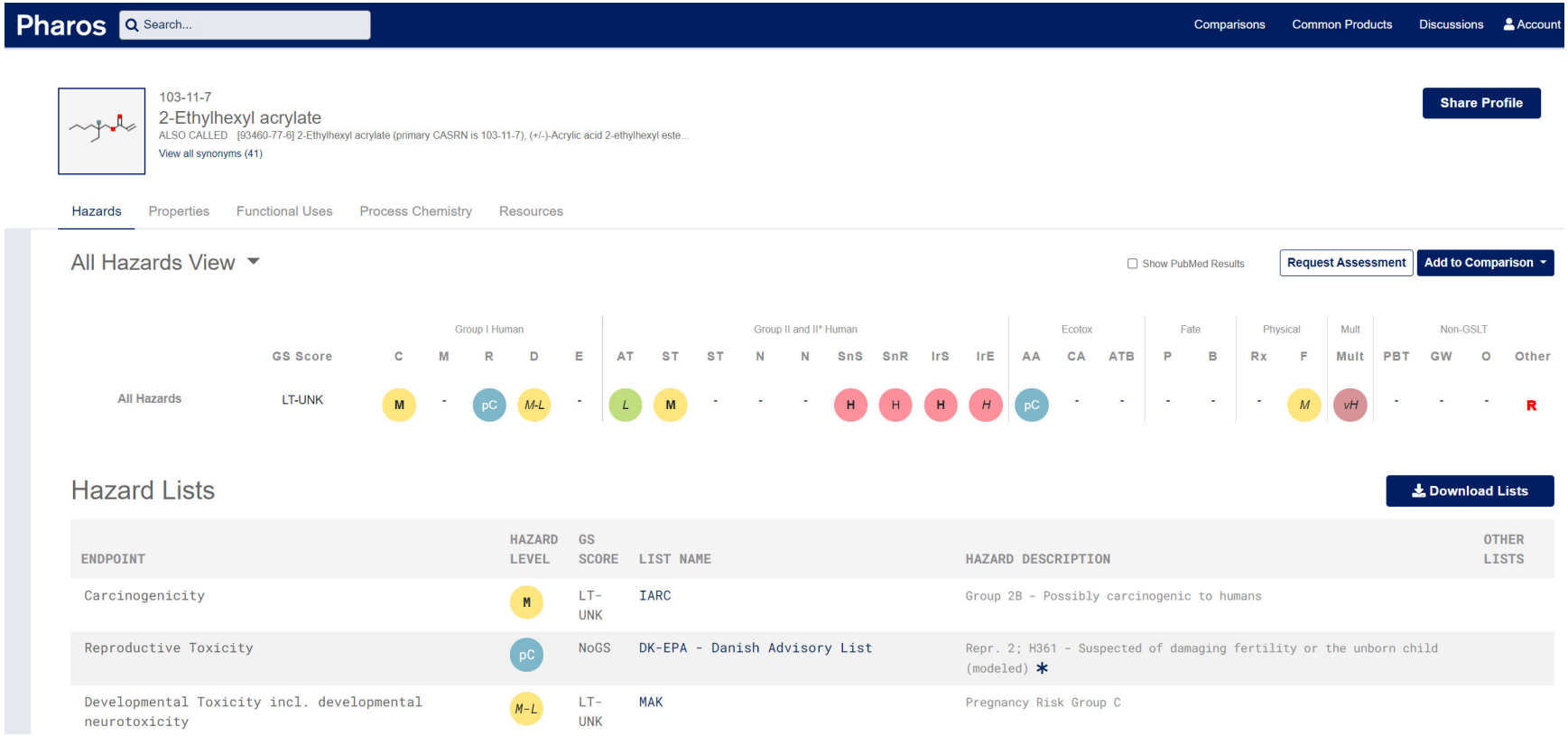
Chemical Name	Final GreenScreen® Benchmark Score
2-EHA	2

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												
2	Yes	Yes	Yes	Yes	Yes							2
3												
4												

APPENDIX C: Pharos Output for 2-EHA (CAS #103-11-7)



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103-11-7
2-Ethylhexyl acrylate
ALSO CALLED [93460-77-6] 2-Ethylhexyl acrylate (primary CASRN is 103-11-7), (+/-)-Acrylic acid 2-ethylhexyl este...
View all synonyms (41)

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All Hazards View

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		Group I Human					Group II and II* Human								Ecotox			Fate		Physical		Mult	Non-GSLT			
	GS Score	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
All Hazards	LT-UNK	M	-	pC	M-L	-	L	M	-	-	-	H	H	H	H	pC	-	-	-	-	-	M	vH	-	-	-	R

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Carcinogenicity	M	LT-UNK	IARC	Group 2B - Possibly carcinogenic to humans	
Reproductive Toxicity	pC	NoGS	DK-EPA - Danish Advisory List	Repr. 2; H361 - Suspected of damaging fertility or the unborn child (modeled) *	
Developmental Toxicity incl. developmental neurotoxicity	M-L	LT-UNK	MAK	Pregnancy Risk Group C	

Acute Mammalian Toxicity	L	LT-UNK	GHS - New Zealand	6.1E (oral) - Acutely toxic	
Systemic Toxicity/Organ Effects-Single Exposure	M	LT-UNK	EU - GHS (H-Statements)	H335 - May cause respiratory irritation [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	+3
	M	LT-UNK	GHS - Australia	H335 - May cause respiratory irritation [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H370 - Causes damage to organs (unverified) [Specific target organ toxicity - single exposure - Category 1]	
Skin Sensitization	H	LT-UNK	MAK	Sensitizing Substance Sh - Danger of skin sensitization	+4
	H-M	LT-UNK	EU - GHS (H-Statements)	H317 - May cause an allergic skin reaction [Skin sensitization - Category 1]	
	H	LT-UNK	GHS - New Zealand	6.5B (contact) - Contact sensitisers (Cat. 1)	
	H-M	LT-UNK	GHS - Australia	H317 - May cause an allergic skin reaction [Skin sensitization - Category 1]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H317 - May cause an allergic skin reaction (unverified) [Skin sensitization - Category 1]	
Respiratory Sensitization	H	NoGS	CHE - Toxicant Database	Asthma - allergen, sensitizer - strong evidence *	

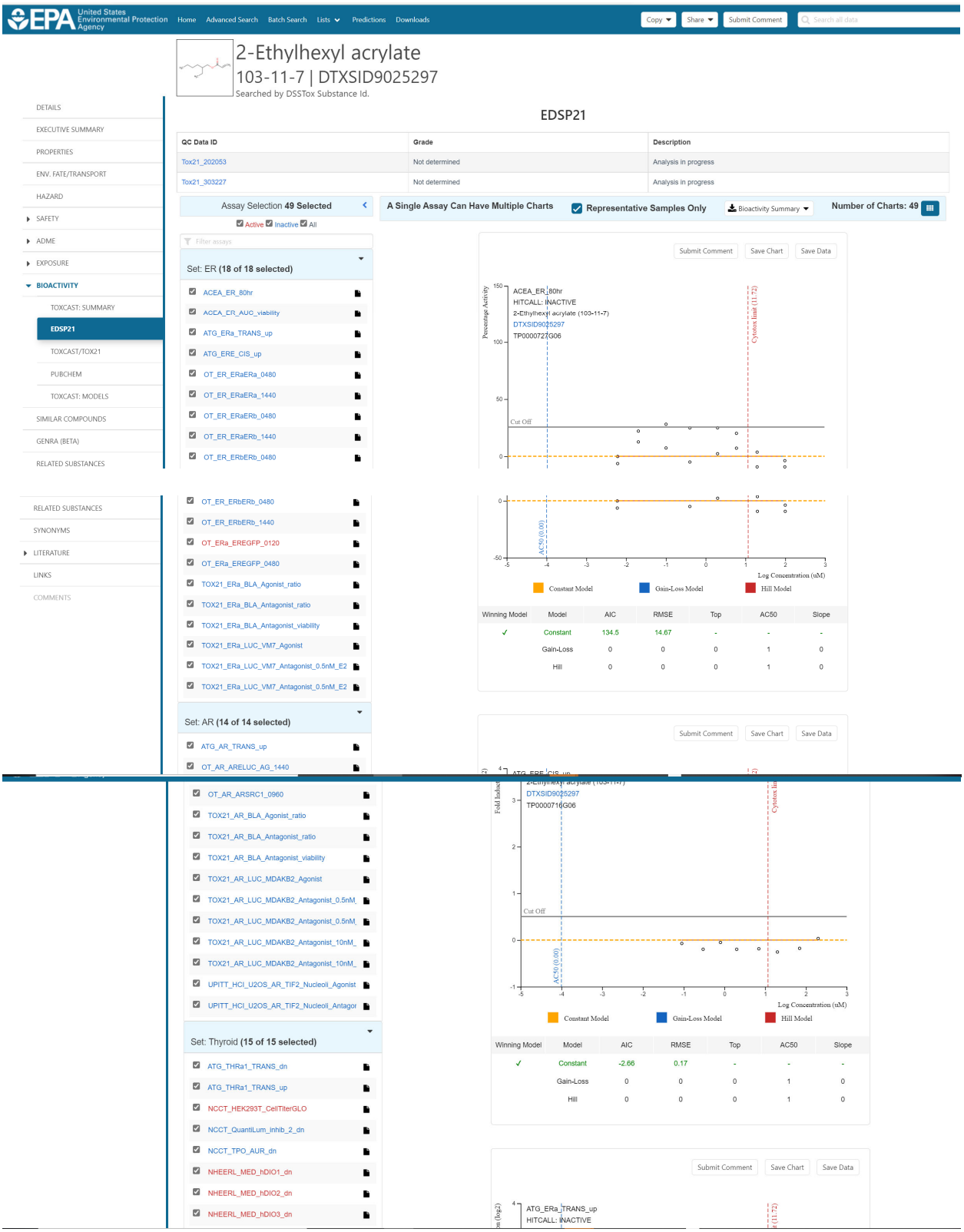
Skin Irritation/Corrosivity	H	LT-UNK	EU - GHS (H-Statements)	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	+4
	H	LT-UNK	GHS - Australia	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	
	H	LT-UNK	GHS - New Zealand	6.3A - Irritating to the skin (Cat. 2)	
	pC	NoGS	DK-EPA - Danish Advisory List	Skin Irrit. 2 - Causes skin irritation (modeled) *	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	
Eye Irritation/Corrosivity	H	LT-UNK	GHS - New Zealand	6.4A - Irritating to the eye (Cat. 2A)	+2
	H-M	LT-UNK	GHS - Japan	H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H318 - Causes serious eye damage (unverified) [Serious eye damage/eye irritation - Category 1]	
Acute Aquatic Toxicity	pC	NoGS	DK-EPA - Danish Advisory List	Aquatic Acute1 - Very toxic to aquatic life (modeled) *	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H401 - Aquatic Acute 2 - Toxic to aquatic life (DELETED EU does not use Cat 2) (unverified) [Hazardous to the aquatic environment (acute) - Category 2]	
Flammability	M	LT-UNK	GHS - New Zealand	3.1D - Flammable Liquids: low hazard	+2
	M	LT-UNK	Québec CSST - WHMIS 1988	Class B3 - Combustible liquids	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H227 - Combustible liquid (unverified) [Flammable liquids - Category 4]	

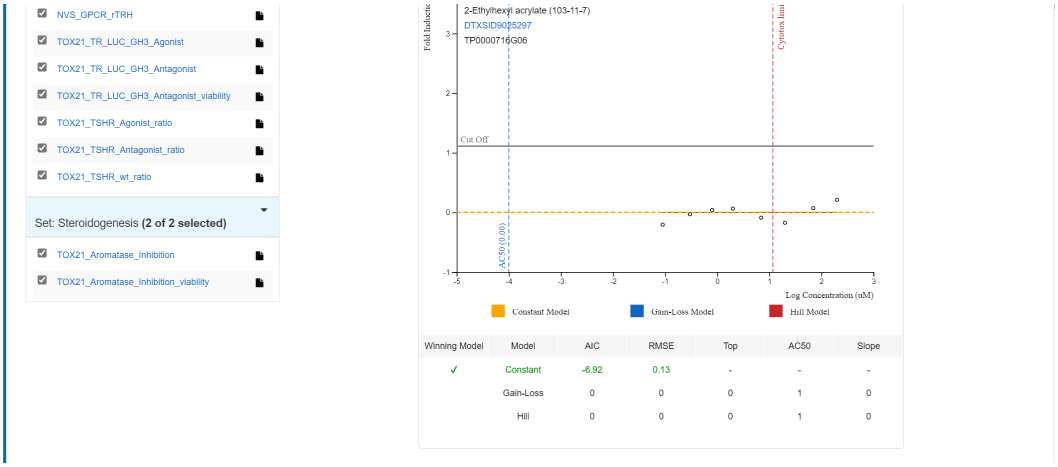
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-UNK	German FEA - Substances Hazardous to Waters	Class 1 - Low Hazard to Waters	
Carcinogenicity,Mutagenicity/Genotoxicity Reproductive Toxicity, Developmental Toxicity, Acute Mammalian Toxicity, or System Toxicity/Organ Effects.	U	LT-UNK	Québec CSST - WHMIS 1988	Class D2B - Toxic material causing other toxic effects	+1
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	vH	LT-UNK	GHS - New Zealand	6.9A (inhalation) - Toxic to human target organs or systems (Cat. 1)	+1
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-UNK	GHS - New Zealand	9.1C (algal) - Harmful in the aquatic environment	+2

Restricted Substance Lists (4)

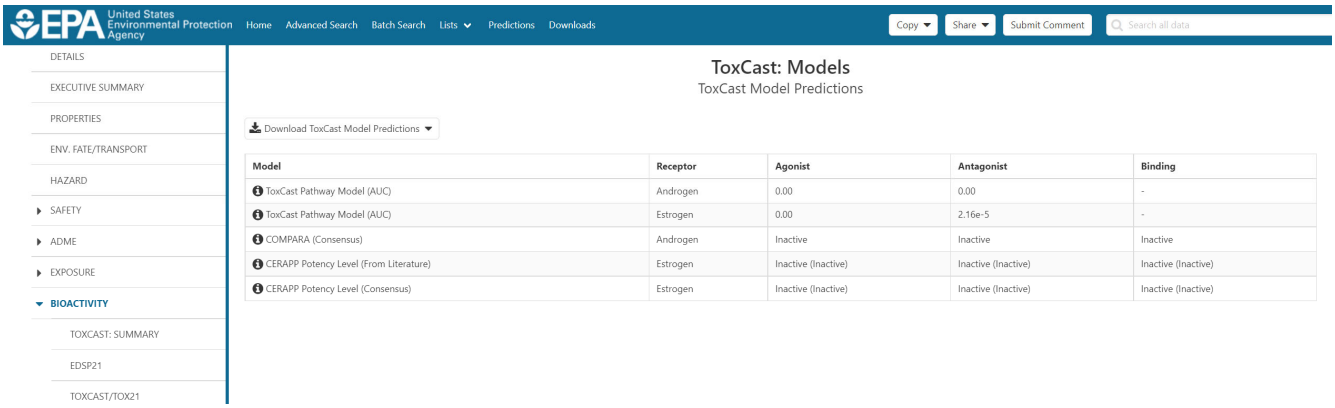
- CA SCP - Candidate Chemicals: Candidate Chemical List
- Credo Beauty's Restricted Substance List: Restricted Chemicals - see Credo for potential source/use restrictions ✱
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- P&W - Precautionary List: Watch List ✱

APPENDIX D: U.S. EPA Bioactivity (EDSP21) Summary for 2-EHA (CAS #103-11-7)



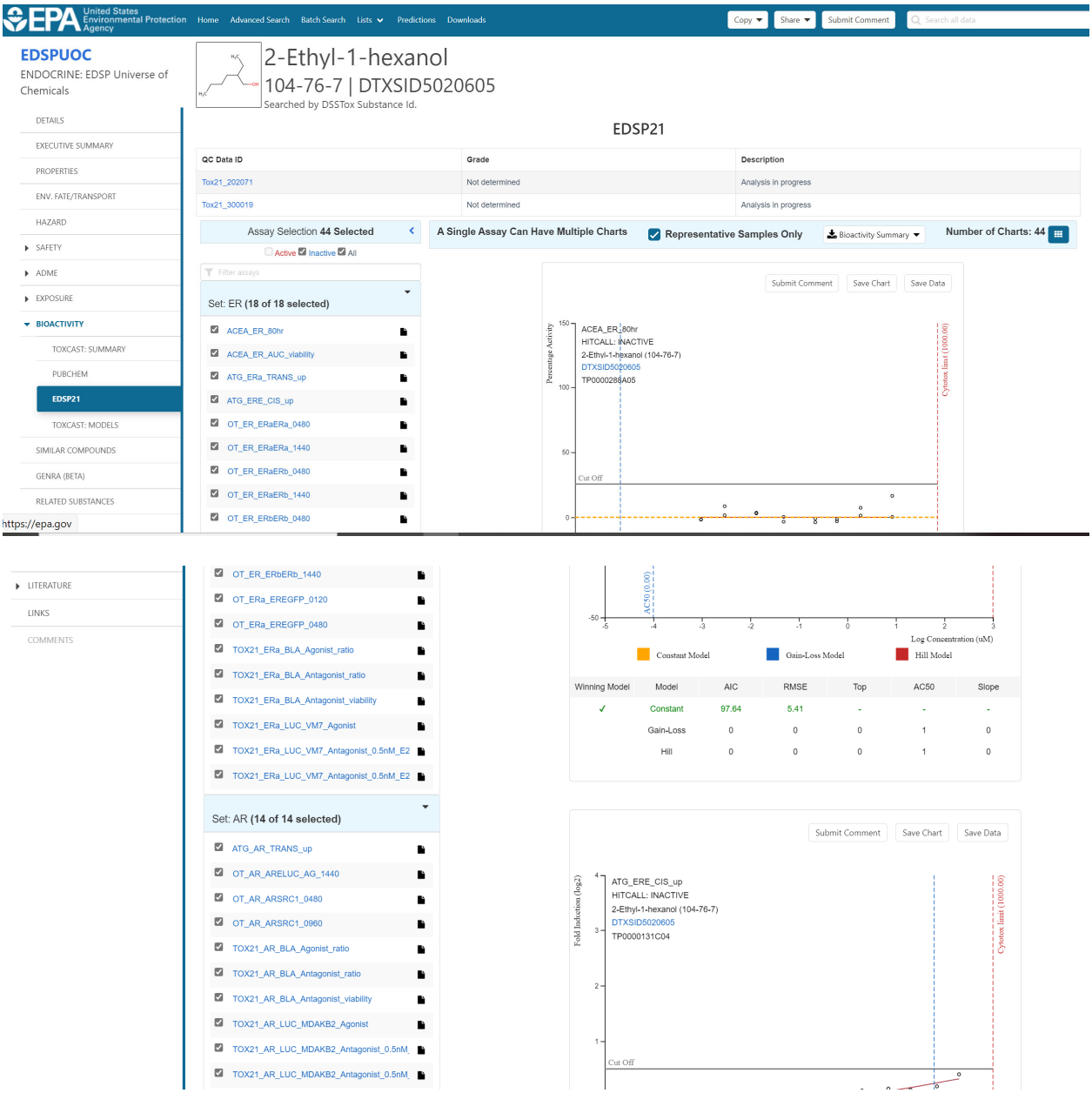


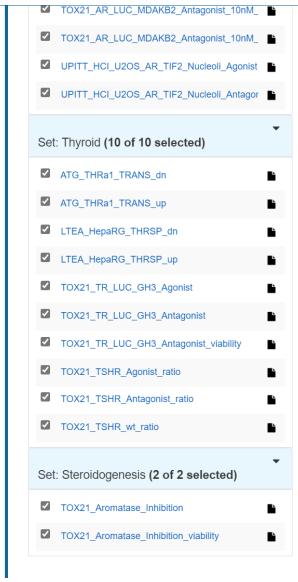
APPENDIX E: U.S. EPA Bioactivity (ToxCast Models) Summary for 2-EHA (CAS #103-11-7)

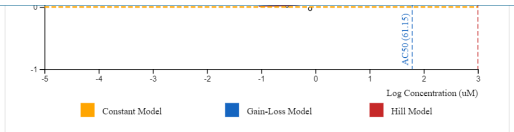


Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.00	-
ToxCast Pathway Model (AUC)	Estrogen	0.00	2.16e-5	-
COMPARA (Consensus)	Androgen	Inactive	Inactive	Inactive
CERAPP Potency Level (From Literature)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)
CERAPP Potency Level (Consensus)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)

APPENDIX F: U.S. EPA Bioactivity (EDSP21) Summary for 2-EH (CAS #104-76-7)



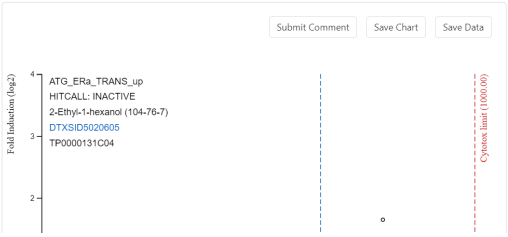




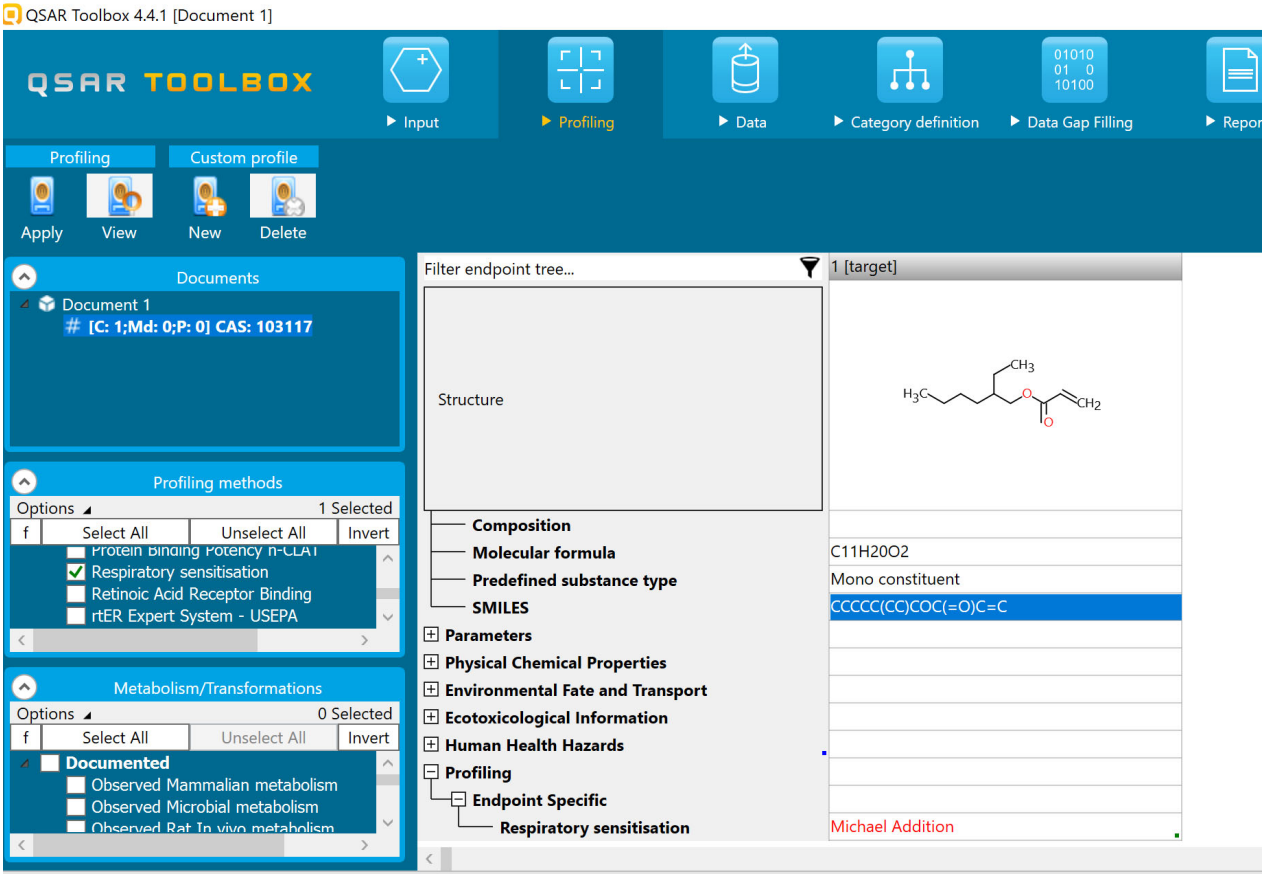
Winning Model	Model	AIC	RMSE	Top	AC50	Slope
	Constant	-2.94	0.18	-	-	-
	Gain-Loss	-9.97	0.06	0.49	61.38	0.58
✓	Hill	-13.97	0.06	0.49	61.15	0.58

Flags

Borderline inactive



APPENDIX G: OECD Toolbox Profile Results for 2-EHA (CAS #103-11-7)



APPENDIX H: EPI Suite™ Modeling Results for 2-EHA (CAS #103-11-7)

(Estimated values included in the GreenScreen® are highlighted and bolded)

CAS Number: 000103-11-7
SMILES : O=C(OCC(CCCC)CC)C=C
CHEM : 2-ETHYLHEXYL ACRYLATE
MOL FOR: C11 H20 O2
MOL WT : 184.28
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 4.64
Boiling Point (deg C) : 213.50
Melting Point (deg C) : -90.00
Vapor Pressure (mm Hg) : 0.178
Water Solubility (mg/L): 9.6
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 4.09

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 216.92 (Adapted Stein & Brown method)
Melting Pt (deg C): -10.43 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 0.183 (Mean VP of Antoine & Grain methods)
VP (Pa, 25 deg C) : 24.4 (Mean VP of Antoine & Grain methods)
MP (exp database): -90 deg C
BP (exp database): 213.5 deg C
VP (exp database): 1.78E-01 mm Hg (2.37E+001 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 8.422
log Kow used: 4.64 (user entered)
melt pt used: -90.00 deg C
Water Sol (Exper. database match) = 100 mg/L (25 deg C)
Exper. Ref: CHEMICALS INSPECTION AND TESTING INSTITU (1992)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 24.593 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Acrylates

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 6.72E-004 atm-m3/mole (6.81E+001 Pa-m3/mole)
Group Method: 6.00E-004 atm-m3/mole (6.08E+001 Pa-m3/mole)
Exper Database: 4.32E-04 atm-m3/mole (4.38E+001 Pa-m3/mole)
For Henry LC Comparison Purposes:

User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 4.496E-003 atm-m3/mole (4.555E+002 Pa-m3/mole)
VP: 0.178 mm Hg (source: User-Entered)
WS: 9.6 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 4.64 (user entered)
Log Kaw used: -1.753 (exp database)
Log Koa (KOAWIN v1.10 estimate): 6.393
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.9424
Biowin2 (Non-Linear Model) : 0.9982
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.2305 (weeks)
Biowin4 (Primary Survey Model) : 4.0799 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.6208
Biowin6 (MITI Non-Linear Model): 0.7551
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.2746
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 23.7 Pa (0.178 mm Hg)
Log Koa (Koawin est): 6.393
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 1.26E-007
Octanol/air (Koa) model: 6.07E-007
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 4.57E-006
Mackay model : 1.01E-005
Octanol/air (Koa) model: 4.85E-005

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 20.1115 E-12 cm3/molecule-sec
Half-Life = 0.532 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 6.382 Hrs
Ozone Reaction:
OVERALL Ozone Rate Constant = 0.175000 E-17 cm3/molecule-sec
Half-Life = 6.549 Days (at 7E11 mol/cm3)
Fraction sorbed to airborne particulates (phi):
7.34E-006 (Junge-Pankow, Mackay avg)

4.85E-005 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 359.5 L/kg (MCI method)
Log Koc: 2.556 (MCI method)
Koc : 2667 L/kg (Kow method)
Log Koc: 3.426 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 1.330E-002 L/mol-sec
Kb Half-Life at pH 8: 1.651 years
Kb Half-Life at pH 7: 16.512 years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 2.728 (BCF = 535.1 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.5935 days (HL = 0.255 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.025 (BCF = 106)
Log BAF Arnot-Gobas method (upper trophic) = 2.025 (BAF = 106)
log Kow used: 4.64 (user entered)

Volatilization from Water:
Henry LC: 0.000432 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 3.225 hours
Half-Life from Model Lake : 149 hours (6.209 days)

Removal In Wastewater Treatment:
Total removal: 66.52 percent
Total biodegradation: 0.53 percent
Total sludge adsorption: 60.21 percent
Total to Air: 5.78 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**2.04**	**11.8**	**1000**
Water	**20.8**	**360**	**1000**
Soil	**76.8**	**720**	**1000**
Sediment	**0.313**	**3.24e+003**	**0**

Persistence Time: 364 hr

Level III Fugacity Model: (MCI Method with Water percents)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	2.04	11.8	1000
Water	20.8	360	1000
water	(20.8)		

biota (0.0453)
suspended sediment (0.0112)
Soil 76.8 720 1000
Sediment 0.313 3.24e+003 0
Persistence Time: 364 hr

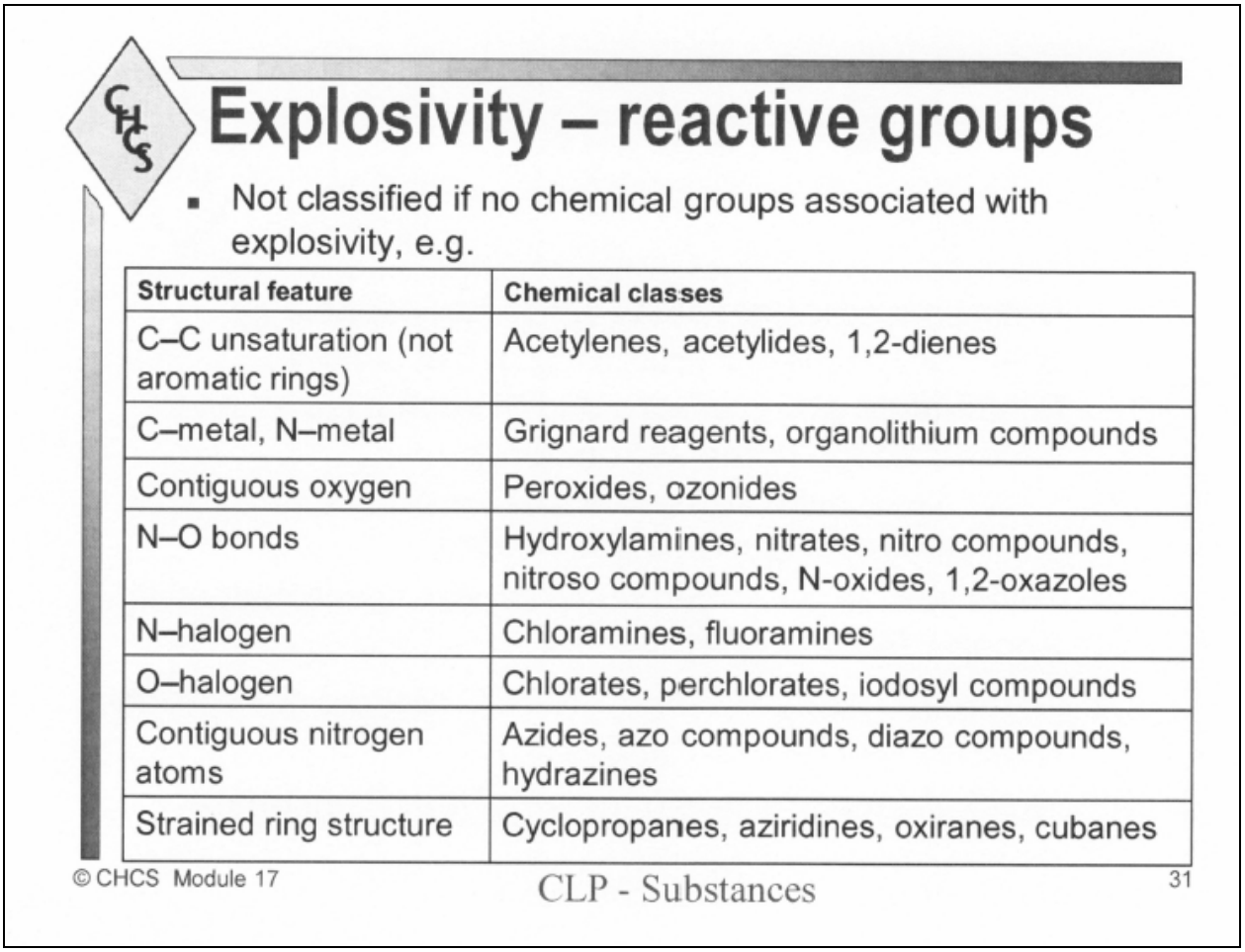
Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.41	11.8	1000
Water	15.8	360	1000
water	(15.3)		
biota	(0.0334)		
suspended sediment	(0.411)		
Soil	74.6	720	1000
Sediment	8.21	3.24e+003	0

Persistence Time: 462 hr

APPENDIX I: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



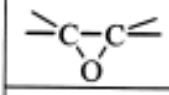
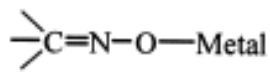
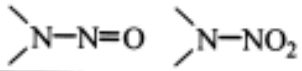
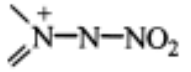
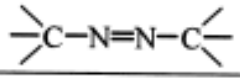
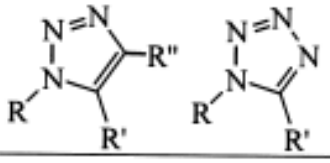
Explosivity – reactive groups

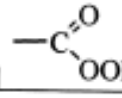
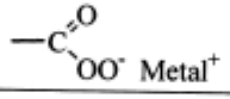
- Not classified if no chemical groups associated with explosivity, e.g.

Structural feature	Chemical classes
C–C unsaturation (not aromatic rings)	Acetylenes, acetylides, 1,2-dienes
C–metal, N–metal	Grignard reagents, organolithium compounds
Contiguous oxygen	Peroxides, ozonides
N–O bonds	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
N–halogen	Chloramines, fluoramines
O–halogen	Chlorates, perchlorates, iodosyl compounds
Contiguous nitrogen atoms	Azides, azo compounds, diazo compounds, hydrazines
Strained ring structure	Cyclopropanes, aziridines, oxiranes, cubanes

Explosivity – Full List

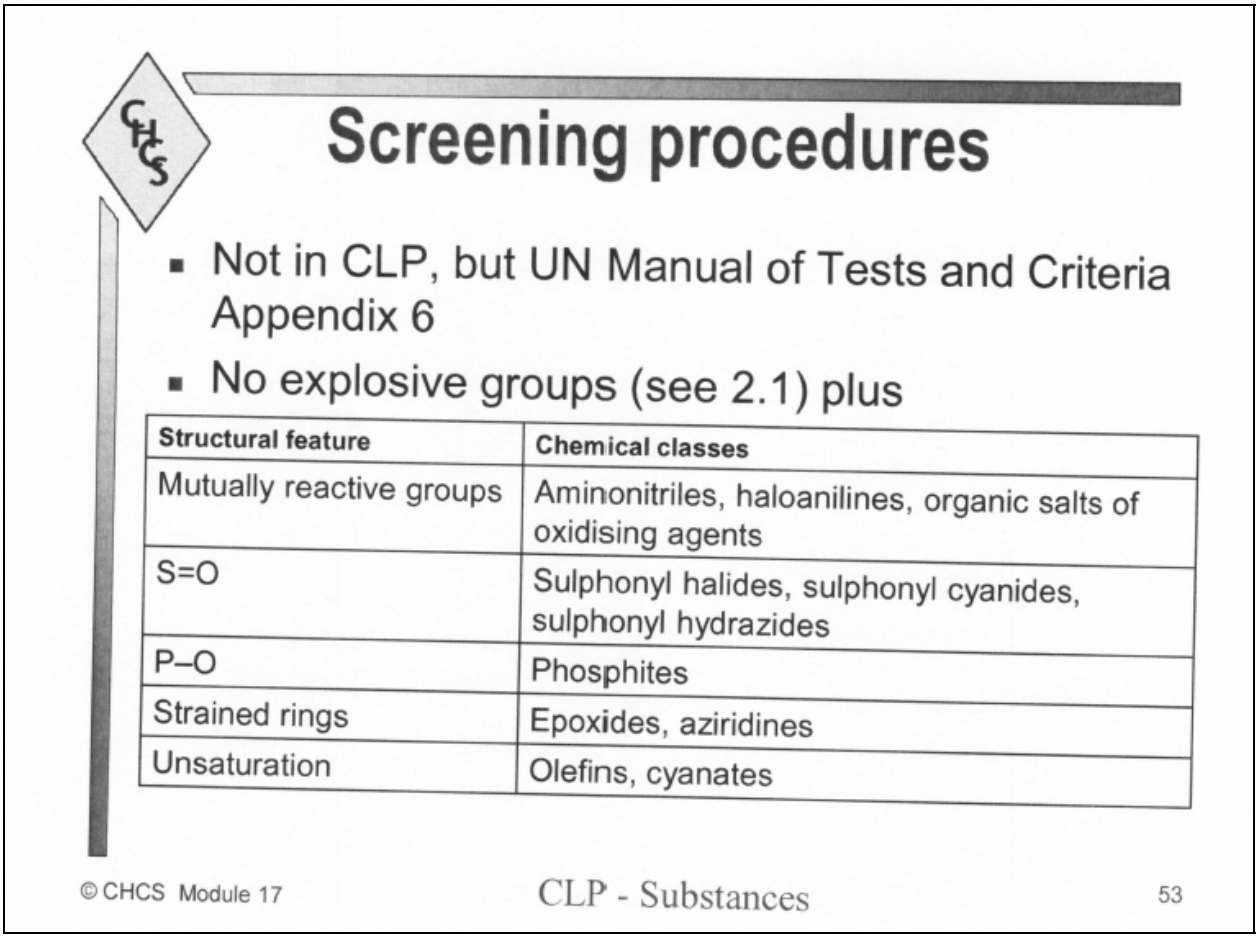
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	Chemical Class
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
	Diazo Compounds
-N=O -NO_2	Nitroso and Nitro Compounds,
R-O-N=O R-O-NO_2	Acyl or Alkyl Nitrites and Nitrates
	1,2-Epoxides
	Metal Fulminates or *aci*-Nitro Salts
	N-Metal Derivatives (especially heavy metals)
	N-Nitroso and N-Nitro Compounds
	N-Azolium Nitroimidates
	Azo Compounds
Ar-N=N-O-Ar	Arene Diazoates
$(ArN=N)_2O$, $(ArN=N)_2S$	Bis-Arenediazo Oxides and Sulfides
RN=N-NR'R''	Triazines
	High-nitrogen Compounds: e.g. Triazoles, Tetrazoles

Chemical group	Chemical Class
[1] ROOR', [2] 	Peroxy Compounds: [1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic); [2] Peroxo acids (R'=H), Peroxyesters (R'=organic)
[1] ROOMetal, [2] 	Metal peroxides, Peroxoacids salts
$-N_3$	Azides e.g. PbN_6, CH_3N_3
$^{-}O-C-N_2^{+}$	Arenediazonium oxides i.e. inner diazonium salts in which the counter ion is an oxide
Ar-N=N-S- Ar-N=N-S-Ar	Diazonium sulfides and derivatives, Arenediazo Aryl Sulfides
XO_n	Halogen Oxide: e.g. percholrates, bromates, etc
NX_3 e.g. NCl_3, $RNCl_2$	N-Halogen Compounds

Adapted from Bretherick (Bretherick's Handbook of Reactive Chemical Hazards 6th Ed., 1999, Butterworths, London)

Self-Reactive Substances



APPENDIX J: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for 2-EA. This GreenScreen® assessment has undergone one round of updates, and the benchmark score remains the same.

Table 5: Change in GreenScreen® Benchmark™ for 2-EA

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
August 18, 2021	BM-2	v. 1.4	New assessment
November 16, 2021	BM-2	v. 1.4	Updated confidence levels of the scores for reproductive toxicity-R and developmental toxicity-D from high to low. Minor updates were made to a few other endpoints without affecting the hazard scores.

Licensed GreenScreen® Profilers

2-EHA GreenScreen® Evaluation Prepared by:

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