SODIUM LEVULINATE

(CAS #19856-23-6)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: November 20, 2023

Expiration Date: November 20, 2028

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GreenScreen® Executive Summary for Sodium Levulinate (CAS #19856-23-6)

Sodium levulinate (IUPAC: sodium 4-oxopentanoate), also known as sodium 4-oxovalerate, is the sodium salt of levulinic acid and will dissociate into levulinate anion in solution. Sodium levulinate functions as a preservative and anti-microbial agent, as a skin conditioning agent in cosmetic formulations, as a chemical intermediate in the manufacturing of biofuels and chemicals, fuel extenders, biodegradable polymers, plasticizers, herbicides, and antibiotics, and as an enzyme inhibitor in pharmaceuticals, and it is used in personal care products and consumer cleaning products. In the United States, the Food and Drug Administration (U.S. FDA) approved levulinic acid as food additive and flavoring agent, and as an inactive ingredient in drug products with a maximum potency level of 16.5 to 20 mg. Sodium levulinate is produced from the reaction of an inorganic base (sodium hydroxide) and levulinic acid, which is biorefined from low-grade cellulose, sugar and starchy crops, wood, organic waste, or algae. Levulinic acid is also found in natural and processed food including Chinese quince, papaya, rice, sake, and wheaten bread. It is a green chemistry alternative to non-renewable energy resources for biomass conversion processes.

Based on decomposition occurring at > 274.6°C and its vapor pressure of 0.0185 Pa (equivalent to 1.39 x 10^{-4} mm Hg, the limit value at which no reproducible or relevant weight loss was found), sodium levulinate is not a volatile organic compound (VOC). It is very soluble in water (797,000 mg/L). Sodium levulinate is not explosive, oxidizing, or flammable.

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

- Benchmark 2f
    - Very High Group II Human Toxicity (eye irritation-IrE)

Data gaps (DG) exist for endocrine activity-E and respiratory sensitization -SnR*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), sodium levulinate meets requirements for a GreenScreen Benchmark™ Score of 3 despite the hazard data gap. In a worst-case scenario, if sodium levulinate 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 silico* modeling for endocrine activity, respiratory sensitization, chronic aquatic toxicity, persistence, and bioaccumulation, and a variety of *in vitro* studies for genotoxicity, skin sensitization, skin irritation, and eye irritation. 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 sodium levulinate's NAMs dataset include limited experimental data for carcinogenicity, endocrine activity, skin and respiratory sensitization, and skin irritation, and lack of established test methods for respiratory sensitization. Sodium levulinate's Type II (extrapolation output) uncertainties include lack of defined applicability domains of OECD QSAR Toolbox and ToxCast models in examination of structural alerts, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions, the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization, and inability of individual *in vitro* skin

sensitization, skin irritation and eye irritation tests to completely differentiate certain GHS categories. Some of sodium levulinate's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for Sodium Levulinate

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*	*	*								
L	**L**	*L*	**L**	DG	**M**	**L**	**L**	*L*	**L**	*M*	DG	*L*	**vH**	**L**	*L*	**vL**	**vL**	*L*	**L**

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 Sodium Levulinate (CAS #19856-23-6)

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

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Deb Remeikas, M.A.
Title: Toxicologist
Organization: ToxServices LLC
Date: October 10, 2023

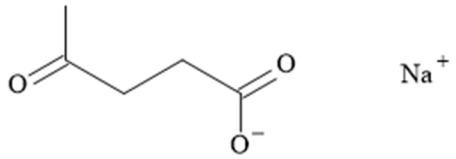
Quality Control Performed By:
Name: Jennifer Rutkiewicz, Ph.D.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: November 20, 2023

Expiration Date: November 20, 2028[2]

Chemical Name: Sodium levulinate

CAS Number: 19856-23-6

Chemical Structure(s):



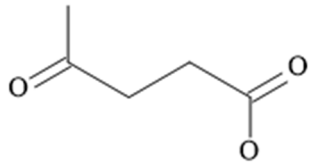
Also called: Levulinic acid, sodium salt; Pentanoic acid, 4-oxo-, sodium salt; Sodium 4-oxopentanoate; Sodium 4-oxovalerate (PubChem 2023).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Sodium levulinate has a limited dataset; therefore, levulinic acid (CAS #123-76-2) was identified as a surrogate. Sodium levulinate is the sodium salt of levulinic acid and will dissociate into levulinate anion in solution. Levulinic acid has maximum common structure (MCS) Tanimoto coefficients of 0.8889 with the target chemical, indicating a high degree of structural similarity (ChemMine 2023, Appendix C). In addition, the authors of the ECHA dossier identified levulinic acid as the read-across substance (ECHA 2023a), and in the evaluation of the REACH dossier, ECHA (2020), notes the read-across substance used for sodium levulinate is levulinic acid; therefore, the surrogate substance "Substance 1" listed in the ECHA dossier is levulinic acid (ECHA 2020).

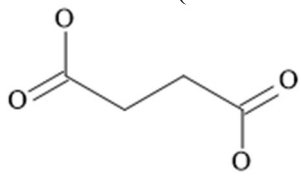
Monosodium succinate (CAS #2922-54-5) and disodium succinate (CAS #150-90-3, CAS #6106-21-4 for the hexahydrate compound), the monosodium and disodium salts of succinic acid (CAS #110-15-6), respectively, were identified as additional surrogates. Succinic acid, a 4-carbon dicarboxylic acid, has maximum common structure (MCS) Tanimoto coefficients of 0.7778 and 0.7000, with levulinic acid, a 5-carbon keto carboxylic acid, and the target chemical, respectively, indicating a high degree of structural similarity (ChemMine 2023, Appendix C).

[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).

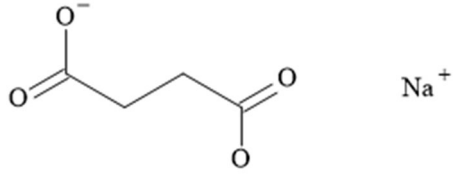
Lastly, due to the limited data on the toxicokinetics of sodium levulinate, levulinic acid, and sodium salts of succinic acid, data on the toxicokinetics of the calcium salt of levulinic acid are included in this assessment because levulinic salts are expected to have similar metabolism and toxicity profiles. ToxServices considered levulinic acid to be a strong surrogate, and prioritized data on levulinic acid. ToxServices considered succinic acid and the sodium salts of succinic acid as weak surrogates, because despite the relatively high Tanimoto coefficients, they differ by the presence of a keto group on sodium levulinate and a second carboxylic acid functional group on the succinates.



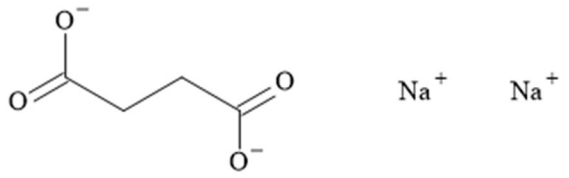
Levulinic acid (CAS #123-76-2)

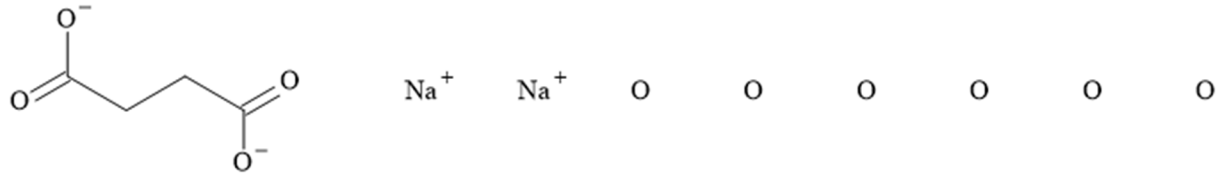


Succinic acid (CAS #110-15-6)

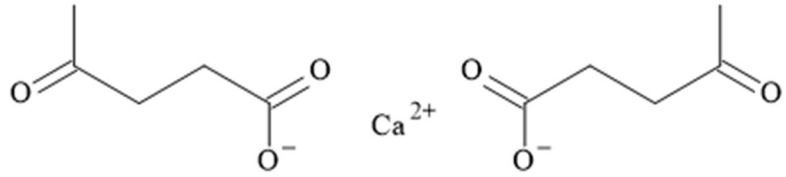


Monosodium salt of succinic acid (CAS #2922-54-5)





Disodium salts of succinic acid (CAS #150-90-3, #6106-21-4 (hexahydrate))



Calcium levulinate (CAS #591-64-0)

Identify Applications/Functional Uses (Danish EPA 2018), EC 2023, CIR 2021, PubChem 2023)**:**
1. Preservative
2. Anti-microbial agent
3. Skin conditioning agent

4. Chemical intermediate
5. Enzyme inhibitor
6. Flavoring agent (levulinic acid)
7. Food additive (levulinic acid)

Known Impurities[3]:
No information is available. The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for Sodium Levulinate[4,5 6,7]: Sodium levulinate 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 combinations:

- Benchmark 2f
    - Very High Group II Human Toxicity (eye irritation-IrE)

Data gaps (DG) exist for endocrine activity-E and respiratory sensitization -SnR*. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), sodium levulinate meets requirements for a GreenScreen Benchmark™ Score of 3 despite the hazard data gap. In a worst-case scenario, if sodium levulinate 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 Sodium Levulinate

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*	*	*								
L	**L**	*L*	**L**	DG	**M**	**L**	**L**	*L*	**L**	*M*	DG	*L*	**vH**	**L**	*L*	**vL**	**vL**	*L*	**L**

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.

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 sodium levulinate is readily biodegradable, it is not expected to have relevant transformation products.

[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.

Introduction

Sodium levulinate is produced from the reaction of an inorganic base (sodium hydroxide) and levulinic acid, which is biorefined from low-grade cellulose, sugar and starchy crops, wood, organic waste, or algae. Levulinic acid is also found in natural and processed food including Chinese quince, papaya, rice, sake, and wheaten bread (CIR 2021). It is a green chemistry alternative to non-renewable energy resources for biomass conversion processes (Hussain et al. 2023).

ToxServices assessed sodium levulinate against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2021).

U.S. EPA Safer Choice Program's Safer Chemical Ingredients List

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2023a). 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).

Sodium levulinate is not listed on the 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 2023) 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 sodium levulinate can be found in Appendix D.

- Sodium levulinate is an LT-UNK chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Sodium levulinate is not listed on the U.S. DOT list.
- Sodium levulinate is on the following list 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 Lazard to Waters

Hazard Statement and Occupational Control

No Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for sodium levulinate; however, it was classified by the authors of the REACH dossier and the majority of notifiers in the C&L inventory, as indicated in Table 1. No general personal protective equipment (PPE) recommendations and no occupational exposure limits (OELs) were identified (ECHA 2023a, BeanTown 2021).

Table 1: GHS H Statements for Sodium Levulinate (CAS #19856-23-6) (ECHA 2023a,b)

H Statement	H Statement Details
H302	Harmful is swallowed.
H318	Causes serious eye damage.

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

Table 1: GHS H Statements for Sodium Levulinate (CAS #19856-23-6) (ECHA 2023a,b)	
H Statement	**H Statement Details**
No harmonized GHS H statements are reported by the European Chemicals Agency (ECHA).	

Physicochemical Properties of Sodium Levulinate

Sodium levulinate is a white, off-white solid powder at standard temperature and pressure. At a vapor pressure of 0.000139 mm Hg (limit value) no relevant or reproducible weight loss was found; therefore, it is expected to exist in the solid and liquid phase. It is very soluble in water (797,000 mg/L). It is more soluble in water than octanol (log K_{ow} of -0.616) at acidic pH, indicating that it is not likely to bioaccumulate in aquatic biota.

Table 2: Physical and Chemical Properties of Sodium Levulinate (CAS #19856-23-6)		
Property	**Value**	**Reference**
Molecular formula	$C_5H_7O_3Na$	ECHA 2023a
SMILES Notation	CC(=O)CCC(=O)[O-].[Na+]	PubChem 2023
Molecular weight	138.1 g/mol	CIR 2021, ECHA 2023a
Physical state	Solid, powder	CIR 2021, ECHA 2023a
Appearance	White, off-white	CIR 2021, ECHA 2023a
Melting point	170.2°C (OECD Guideline 102, EU Method A.1)	CIR 2021, ECHA 2023a
Boiling point	Not observed, decomposition at > 274.6°C (OECD Guideline 103, EU Method A.2)	CIR 2021, ECHA 2023a
Vapor pressure	0.000139 mm Hg at 135°C (limit value – no relevant and reproducible weight loss) (OECD Guideline 104/EU Method A.4)	CIR 2021, ECHA 2023a
Water solubility	797,000 mg/L at 20°C, pH = 8 (OECD Guideline 105, EU Method A.6)	CIR 2021, ECHA 2023a
Dissociation constant	pKa = 9.38 at 25°C (exp)	CIR 2021, ECHA 2023a
Density/specific gravity	1.4795 g/mL at 20°C (OECD Guideline 109, EU Method A.3)	CIR 2021, ECHA 2023a
Partition coefficient	-0.616 at 20°C, pH = 2.07 (OECD Guideline 107, EU Method A.8)	CIR 2021, ECHA 2023a

Toxicokinetics

Limited data are available on the toxicokinetics of sodium levulinate, levulinic acid, and sodium salts of succinic acid; however, data were identified for the calcium salt of levulinic acid, identified as levulinate. Absorption, distribution, metabolism, and excretion (ADME) studies were performed through *in vitro* study of rat livers exposed to levulinate, intravenous exposure of radiolabeled (^{13}C) levulinate in rats, oral exposure in rats, and in children receiving intravenous doses of calcium levulinate (Kølvraa et al. 1977, Harris et al. 2010, 2013, CIR 2021).

- *Absorption:* No data on the absorption of sodium levulinate or levulinic acid were identified for the dermal and inhalation exposure routes. Studies of rats that received oral doses of calcium levulinate determined that it was absorbed and distributed to plasma and organs, but absorption rates were not reported (Harris et al. 2013).
- *Distribution:* Levulinate was found in the plasma, brain, and livers of rats administered oral doses of 2 mmol/kg calcium levulinate via gavage; additional tissues were not evaluated. The combined peak concentration of levulinate metabolites in the liver was 645 nmol/g dry wt at 45 minutes post exposure (Harris et al. 2013).

- *Metabolism: In vitro* and an *in vivo* oral and intravenous studies in rats found metabolism of levulinate occurs in the liver, and other unspecified organs. Levulinate is reduced by a cytosolic and mitochondrial dehydrogenase to (R)-4 and to (S)-4-hydroxypentanoate by a mitochondrial dehydrogenase or the racemase metabolic pathway. Levulinate is ultimately catabolized by three parallel pathways into propionyl-COA, acetyl-COA, and lactate as shown in Figure 2, below (Harris et al. 2011, 2013).
- *Excretion:* When levulinate was administered to 5 children intravenously at unspecified pharmacological doses, 3.5 – 11.0 mg levulinic acid and 4.5-10.4 mg 4-hydroxypentanoic acid, the metabolite of levulinic acid, were excreted in urine during the 24-hour period post treatment (Kølvraa et al. 1977).

Overall, levulinic acid and its salts are expected to be absorbed via the oral routes, with a lack of data for the dermal and inhalation routes. It is then distributed throughout the body and has been detected in the brain, liver, and blood, but comprehensive tissue analyses have not been performed to fully characterize the distribution. Levulinate is metabolized into 4-hydroxypentanote by mitochondrial hydrogenase and/or via catabolic pathways, ultimately metabolized into propionyl-COA, acetyl-COA, and lactate (important in cellular processes), and excreted in the urine as unchanged levulinate or its metabolite, 4-hydroxypentanone.

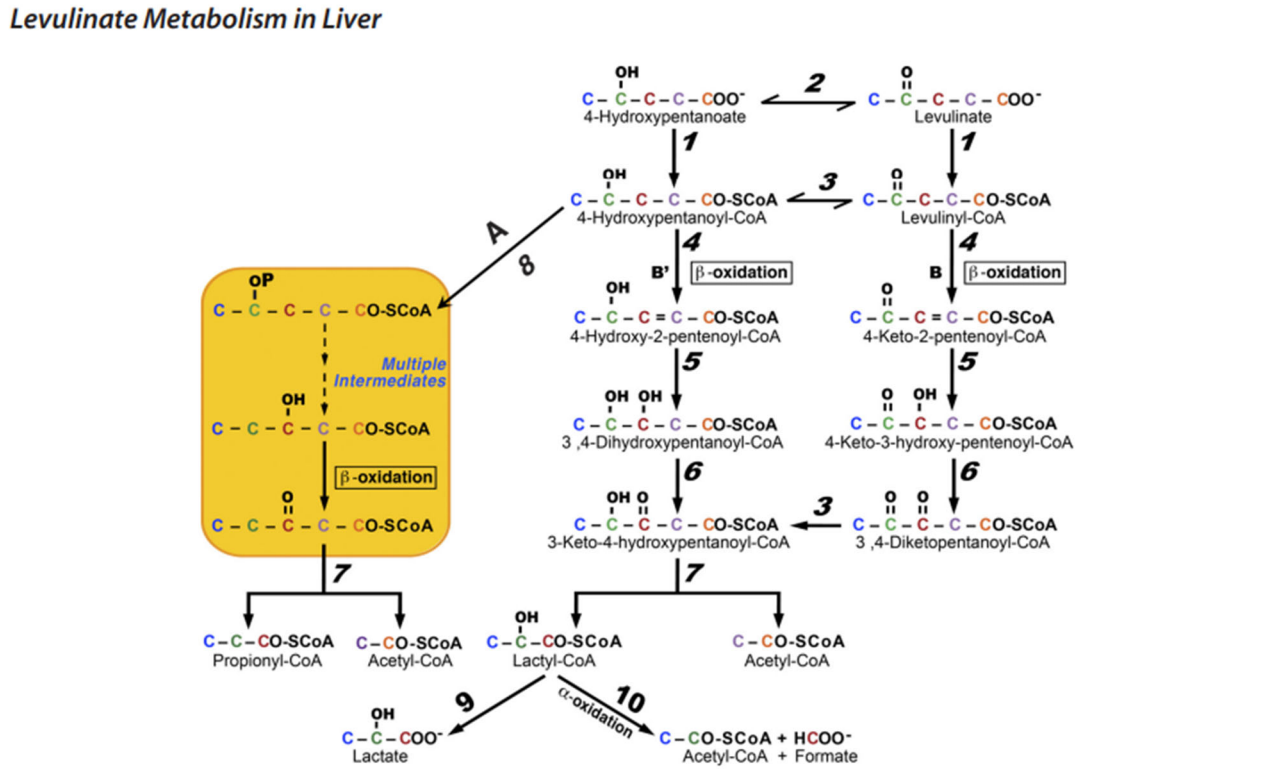


FIGURE 1. **Proposed scheme for the metabolism of levulinate and 4-hydroxypentanoate.** The hypothetical enzyme activities, designated by numbers in *italics* are: ***1,*** acid-CoA ligase; ***2***, hydroxyacid dehydrogenase; ***3,*** 4-hydroxyacyl-CoA dehydrogenase; ***4,*** acyl-CoA dehydrogenase; ***5,*** enoyl-CoA hydratase; ***6,*** 3-hydroxyacyl-CoA dehydrogenase; ***7,*** 3-ketoacyl-CoA thiolase; ***8,*** 4-hydroxyacyl-CoA kinase; ***9,*** acyl-CoA hydrolase; ***10,*** α-oxidation enzymes. The "multiple reactions" mentioned between 4-phosphopentanoyl-CoA and 3-hydroxypentanoyl-CoA result in the isomerization of 4-hydroxypentanoyl-CoA to 3-hydroxypentanoyl-CoA (5).

Figure 2: Proposed Metabolic Pathways for Levulinate (Harris et al. 2011)

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *L*
Sodium levulinate was assigned a score of Low for carcinogenicity based on a lack of carcinogenicity reported in a 2-year combined systemic toxicity and carcinogenicity drinking water study in rats exposed to the surrogate monosodium succinate. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is low based on measured data for a weak surrogate.

- 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 carcinogenicity studies were identified for sodium levulinate or levulinic acid.
- CCRIS 1997, Maekawa et al. 1990
  - *Surrogate: Monosodium succinate (CAS #2922-54-5)*: There was no evidence of carcinogenicity or non-cancer systemic toxicity in a two-year study with male and female Fischer 344 rats (50/sex/dose) provided drinking water containing 1 or 2% monosodium succinate. The study authors concluded up to 2% monosodium succinate did not elicit toxic or carcinogenic activity in rats. Thus, ToxServices identified the NOAEL for this study is 2%. Using default water factors for male and female F344 rats in a chronic study (TERA Undated), the equivalent daily intakes are: 20,000 mg/L water * 0.129 L water/kg bw/day = 2,580 mg/kg/day (males) and 20,000 mg/L water * 0.144 = 2,880 mg/kg bw/day (females).

Mutagenicity/Genotoxicity (M) Score (H, M, or L): L
Sodium levulinate was assigned a score of Low for mutagenicity/genotoxicity based on negative results for mutagenicity and clastogenicity in bacterial reverse mutation assays, an *in vitro* mammalian cell mutagenicity assay, and an *in vitro* chromosome aberration assay for the surrogate levulinic acid. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high based on high quality, reliable data on a strong surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- CIR 2021
  - *In vitro: Surrogate: Levulinic acid (CAS #123-76-2)*: Levulinic acid was negative for mutagenicity in a bacterial reverse mutation assay conducted according to OECD Guideline 471 using *Salmonella typhimurium* test strains TA98, TA100, and TA1537 and *Escherichia coli* strain WP2 uvrA at concentrations up to 5,000 µg/plate without and with metabolic activation. No increase in the frequency of revertants was observed in any strain at any dose in the presence or absence of metabolic activation. No further details were provided.
  - *In vitro: Surrogate: Levulinic acid (CAS #123-76-2)*: Levulinic acid was not genotoxic in a BlueScreen assay (an assay that measures genotoxic stress through human-derived gene expression) with and without metabolic activation. No additional information was provided.
- ECHA 2023a,c
  - *In vitro: Surrogate: Levulinic acid (CAS #123-76-2)*: Levulinic acid was negative for mutagenicity in a GLP-compliant bacterial reverse mutation assay conducted according to

OECD Guideline 471/EU Method B.13/14 using *Salmonella typhimurium* test strains TA98, TA100, TA102, TA1535 and TA1537 at concentrations up to 5 µL/plate without and with metabolic activation. No precipitation or cytotoxicity were observed, but the study tested up to the recommended limit concentrations. Positive (i.e., sodium azide, benzo(a)pyrene, mitomycin C, 4-nitro-o-phenylenediamine, and 2-aminoanthracene) and negative controls were valid. No increase in the frequency of revertants was observed in any strain at any dose in the presence or absence of metabolic activation (Klimisch 1, reliable without restriction) (Unnamed study 2021, 003 Key, ECHA, CAS #19856-23-6, 2023).

- *In vitro:* *Surrogate: Levulinic acid (CAS #123-76-2)*: Levulinic acid was negative for mutagenicity in a GLP-compliant *in vitro* mammalian gene mutation assay conducted according to OECD Guideline 476 using Chinese hamster V79 cells at concentrations up to 1,160 µg/mL with and without metabolic activation. No precipitation or cytotoxicity were observed. Positive (i.e., 7,12-dimethylbenzanthracene and ethylmethanesulphonate) and vehicle controls were valid. No increase in the mutation frequency was observed with treatment in the presence or absence of metabolic activation (Klimisch 1, reliable without restriction) (Unnamed study 2017, 002 Key, ECHA, CAS #19856-23-6, 2023).
- *In vitro:* *Surrogate: Levulinic acid (CAS #123-76-2)*: Levulinic acid was negative for clastogenicity in a GLP-compliant *in vitro* chromosome aberration study conducted according to OECD Guideline 473 using human lymphocytes at concentrations up to 1,160 µg/mL with and without metabolic activation. No precipitation or cytotoxicity were observed. Positive (i.e., cyclophosphamide and mitomycin C) and negative controls were valid. No evidence of clastogenicity was observed with treatment in the presence or absence of metabolic activation (Klimisch 1, reliable without restriction) (Unnamed study 2016, 001 Key, ECHA, CAS #19856-23-6, 2023).

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

Sodium levulinate was assigned a score of Low for reproductive toxicity based on a NOAEL of 600 mg/kg/day, the highest dose tested, in an OECD Guideline 422 combined repeated dose toxicity study with the reproduction / developmental toxicity screening test with male and female Crj:CD (SD) IGS rats exposed to the surrogate disodium succinate. 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). The confidence in the score is reduced as it is based on a weak surrogate and a screening study that may not have examined all relevant endpoints.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2003, ECHA 2023d
  - *Surrogate: Disodium succinate (CAS #150-90-3)*: In an OECD Guideline 422 combined repeated dose toxicity with the reproduction/developmental toxicity screening test (GLP-unspecified), Crj:CD (SD) IGS rats were exposed to disodium succinate hexahydrate (purity unspecified) in water at 0, 100, 300, or 1,000 mg/kg/day (equivalent to 60, 180, and 600 mg/kg disodium succinate as identified by UNEP) by gavage from 14 days before mating to termination for a total of 52 days for males, and through mating, gestation, and until lactation day 4 for females. There were no mortalities and no treatment related effects reported on clinical signs, reproductive parameters (i.e., estrous cycle, sperm measures, copulation index and fertility index, gestation duration), and pathology of reproductive organs. However, urinary protein was elevated in male parental animals in the top two dose groups and blood urea nitrogen was increased in high-dose female parental animals.

Accordingly, authors of the ECHA dossier established a reproductive NOAEL of 1,000 mg/kg/day (equivalent to 600 mg/kg/day disodium succinate), the highest dose tested (Klimisch 2, reliable without restriction) (UNEP 2003, NITE 2018, 001 Weight of Evidence (WOE), ECHA, CAS #150-90-3, 2023).

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): L
Sodium levulinate was assigned a score of Low for reproductive toxicity based on a NOAEL of 600 mg/kg/day, the highest dose tested, in an OECD Guideline 422 combined repeated dose toxicity study with the reproduction / developmental toxicity screening test with male and female Crj:CD (SD) IGS rats exposed to the surrogate disodium succinate, and a developmental NOAEL of 1,000 mg/kg/day, the highest dose tested, in the presence of maternal toxicity (NOAEL of 500 mg/kg/day based on reduction in body weight and weight gain and food consumption) in an OECD Guideline 414 prenatal toxicity study in Wistar rats exposed to the surrogate levulinic acid. 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). The confidence in the score is high based on high quality, measured data for a strong surrogate with support from a weak surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- CIR 2021, ECHA 2023c
  - *Surrogate: Levulinic acid (CAS #123-76-2)*: In a GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant Wistar rats (20-22/dose) were administered levulinic acid (purity unspecified) in water at doses of 0, 100, 500, and 1,000 mg/kg/day for 15 days, presumably on gestation days 5 to 20, via gavage. No treatment related effects were reported for maternal parameters including mortality, clinical signs of toxicity, thyroid parameters (i.e., serum levels of thyroid hormones, T3, T4, and TSH), behavior, organ weights (uterus and thyroid), histopathology of the thyroid, and gross pathology. Significant reductions in body weight and weight gain were reported for high-dose females on day 14 and 20, which correlated to a reduction in food consumption for the high-dose group on gestation days 8 to 14. A reduction in absolute uterus weight was reported for high-dose females; however, the relative uterus weight was comparable to controls and thus this effect was considered due to the lower body weight at necropsy. For offspring, no treatment related effects were reported for developmental (i.e., number of corpora lutea, number of implantations, pre- and post-implantation loss, number of resorptions, live and total number of delivered offspring, anogenital distance) and external, skeletal, and visceral examinations. Although a reduction in mean fetal body weight was reported for the mid- and high-dose groups, the study authors did not consider these effects treatment-related due to the lack of statistical significance and dose-dependence. Lastly, increased incidence of delayed ossification of cranial bones (i.e., frontal bone, arcus zygomaticus, and nasal bone) was reported for mid- and high-dose offspring; however, the study authors did not consider these findings to be adverse due to the lack of significance and dose dependence in addition to reversibility of these effects during the post-natal period. The study authors established a maternal toxicity NOAEL of 500 mg/kg/day based on reduced body weight and weight gain and food consumption, and a developmental toxicity NOAEL of 1,000 mg/kg/day, the highest dose tested, based on a lack of adverse effects on offspring (Klimisch 1, reliable without restriction) (Unnamed study 2021, Key, ECHA, CAS #123-76-2, 2023).
- UNEP 2003, ECHA 2023d

- *Surrogate: Disodium succinate (CAS #150-90-3)*: In the previously described OECD Guideline 422 combined repeated dose toxicity with the reproduction/developmental toxicity screening test (GLP-unspecified), Crj:CD (SD) IGS rats were exposed to disodium succinate hexahydrate (purity unspecified) in water at 0, 100, 300, or 1,000 mg/kg/day (equivalent to 60, 180, and 600 mg/kg disodium succinate as identified by UNEP) by gavage from 14 days before mating to termination for a total of 52 days for males, and through mating, gestation, and until lactation day 4 for females. There were no mortalities and no adverse treatment related effects reported on clinical signs, developmental parameters (i.e., delivery index, implantation index, parturition index, live birth index, sex ratio and viability index of offspring on day 4 of lactation), and external examination of offspring. Parental body weight was significantly reduced on lactation days 0 and 4 for males, and on day 4 in low-dose females and mid-dose males. These changes on body weight were not considered treatment related by the study authors. Accordingly, authors of the ECHA dossier established a maternal and developmental toxicity NOAEL of 1,000 mg/kg/day (equivalent to 600 mg/kg/day disodium succinate), the highest dose tested (Klimisch 2, reliable without restriction) (UNEP 2003, NITE 2018, 001 Weight of Evidence (WOE), ECHA, CAS #150-90-3, 2023).

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

Sodium levulinate was assigned a score of Data Gap for endocrine activity based on a lack of sufficient data identified for this endpoint. No endocrine effects (T3/T4/TSH, thyroid organ weight, and pathology) were found in parents in an oral prenatal toxicity study surrogate levulinic acid, and modeling for the target chemical did not identify any endocrine activity concerns. Due to insufficient *in vivo* data for all relevant endocrine pathways (i.e., estrogen agonism and antagonism, androgen agonism and antagonism, thyroid, and steroidogenesis), a Data Gap was assigned.

- 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 2023b
  - Sodium levulinate was not evaluated as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century; however, the ToxCast CERAPP Potency Level model predicted sodium levulinate was inactive for estrogen receptor agonist, antagonist, and binding (Appendix E).
- DTU 2023
  - Modeling in the Danish QSAR database provides the following results that are within the applicability domains of the models (Appendix F):
    - Sodium levulinate is predicted to be negative for estrogen receptor α binding (full training set and balanced training set, human *in vitro*), estrogen receptor α activation (human *in vitro*), and androgen receptor inhibition (human *in vitro*) by the model battery consisting of CaseUltra, Leadscope, and SciQSAR models;
    - Sodium levulinate is predicted to be negative for estrogen receptor activation (CERAPP data *in vitro*) and for androgen receptor binding, inhibition and activation (CoMPARA data *in vitro*) and thyroperoxidase (TPO) inhibition (QSAR1 and QSAR2, rat *in vitro*) by the Leadscope models.
- CIR 2021, ECHA 2023c
  - *Surrogate: Levulinic acid (CAS #123-76-2)*: In the previously described GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant Wistar rats (20-22/dose) were administered levulinic acid (purity unspecified) in

water at doses of 0, 100, 500, and 1,000 mg/kg/day on gestation days 5 to 20 via gavage. No treatment related effects were reported for anogenital distance, thyroid parameters (i.e., serum levels of thyroid hormones, T3, T4, and TSH), organ weights (uterus and thyroid), histopathology of the thyroid, and gross pathology (Klimisch 1, reliable without restriction) (Unnamed study 2021, Key, ECHA, CAS #123-76-2, 2023).

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): M

Sodium levulinate was assigned a score of Moderate for acute toxicity based on oral LD_{50} values as low as > 300 to < 2,000 mg/kg in rats for the surrogate levulinic acid. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when oral LD_{50} values are between 300 and 2,000 mg/kg (CPA 2018b). Confidence in the score is high because it is based on measured data on a strong surrogate.

- 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 2023a,c
  - *Oral: Surrogate: Levulinic acid (CAS #123-76-2)*: LD_{50} (female Sprague Dawley rats) > 300 to < 2,000 mg/kg (GLP-compliant, OECD Guideline 423/EU Method B.1 tris) (Klimisch 1, reliable without restriction) (Unnamed study 2016, 001 Key, ECHA, CAS #19856-23-6, 2023).
  - *Dermal: Surrogate: Levulinic acid (CAS #123-76-2)*: LD_{50} (male and female Sprague Dawley rats) > 2,000 mg/kg (GLP-compliant, OECD Guideline 402/EU Method B.3/EPA OPPTS 870.1200) (Klimisch 1, reliable without restriction) (Unnamed study 2017, 001 Key, ECHA, CAS #19856-23-6, 2023).
- CIR 2021
  - *Oral: Surrogate: Levulinic acid (CAS #123-76-2)*: LD_{50} (rats, sex and strain unspecified) = 1,850 mg/kg.
  - *Dermal: Surrogate: Levulinic acid (CAS #123-76-2)*: LD_{50} (rabbits, sex and strain unspecified) > 5,000 mg/kg.

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

Sodium levulinate was assigned a score of Low for systemic toxicity (single dose) based on the lack of systemic toxicity in acute oral and dermal toxicity studies with the surrogate levulinic acid. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (single dose) when adequate data are available and negative, and they are not classified per GHS (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data on a strong surrogate.

- 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 2023a
  - *Oral: Surrogate: Levulinic acid (CAS #123-76-2)*: In the previously described GLP-compliant acute oral toxicity study conducted according to OECD Guideline 423/EU

Method B.1 tris that reported an LD_{50} value of > 300 to < 2,000 mg/kg, female Sprague Dawley rats were administered single oral doses of 300 or 2,000 mg/kg (n= 3/dose) levulinic acid (purity unspecified) in water and observed up to 14 days. All high dose animals were found dead on day 2, and on the day of dosing clinical signs were reported including hunched posture, piloerection, and decreased activity. Due to loss of the animals in the high dose group, following the same study design, a second 300 mg/kg treatment group was treated and observed. At 300 mg/kg, no mortalities and no treatment related effects were reported on clinical signs of toxicity. No abnormal findings were reported at necropsy for both the 300 mg/kg and 2,000 mg/kg treatment groups. Body weight measurements were performed, but no details were provided; however, the study authors indicated there were no signs of toxicity (Klimisch 1, reliable without restriction) (Unnamed study 2016, 001 Key, ECHA, CAS #19856-23-6, 2023).

- *Dermal: Surrogate: Levulinic acid (CAS #123-76-2)*: In the previously described GLP-compliant dermal acute toxicity study conducted according to OECD Guideline 402/EU Method B.3/EPA OPPTS 870.1200 that reported an LD_{50} value > 2,000 mg/kg, male and female Sprague Dawley rats (5/sex) were administered topical applications of 2,000 mg/kg undiluted levulinic acid (purity unspecified) for 24 hours under semiocclusive conditions and followed by a 14-day observation period. No treatment related effects on mortality, clinical signs of toxicity, or gross pathology at necropsy were reported. Body weight measurements were performed, but no details were provided; however, the study authors indicated there were no signs of toxicity (Klimisch 1, reliable without restriction) (Unnamed study 2017, 001 Key, ECHA, CAS #19856-23-6, 2023).

- Limited information was provided for the remaining oral acute toxicity studies identified by UNEP and CIR; therefore, only the key, GLP-compliant, guideline study above was used to evaluate the oral route of exposure for this endpoint due to its higher reliability.

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

Sodium levulinate was assigned a score of Low for systemic toxicity (repeated dose) based on a NOAEL of 1,000 mg/kg/day, the highest dose tested, in an OECD Guideline 408 study in rats exposed to the surrogate levulinic acid. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when oral LOAEL values are greater than 100 mg/kg/day for 90-day studies (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for a strong surrogate.

- 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 2023a,c
  - *Oral: Surrogate: Levulinic acid (CAS #123-76-2)*: In a GLP-compliant repeated dose toxicity study conducted according to OECD Guideline 408/EU Method B.26, male and female Wistar rats (10/sex/basic group, 6/sex/satellite group) were administered 0, 100, 500, or 1,000 mg/kg/day levulinic acid (purity not specified) in water via gavage, 7 days per week, for 90 days. No significant treatment related mortalities and no adverse treatment related effects were reported for clinical signs of toxicity, body weight and weight gain, food consumption and food conversion, water consumption, ophthalmological examination, hematology, clinical chemistry, urinalysis, neurobehavioral examination, absolute and relative organ weights, gross pathology, and histopathology. Incidental and sporadic findings were either found in controls, did not show dose-dependence, are common to the

age, sex, and species of the test animals, and/or were inconsistent without findings in relating parameters. The study authors established a NOAEL of 1,000 mg/kg/day, the highest dose tested, based on a lack of adverse effects (Klimisch 1, reliable without restriction) (Unnamed study 2021, Key, ECHA, CAS #19856-23-6, 2023).

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *L*
Sodium levulinate was assigned a score of Low for neurotoxicity (single dose) based on a lack of signs of neurotoxicity in a standard acute oral and dermal toxicity study in rats exposed to the surrogate levulinic acid receiving non-lethal doses of 300 mg/kg (the highest non-lethal dose) and 2,000 mg/kg, respectively. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (single dose) when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is reduced as there were no specific neurotoxicity examinations.

- 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 2023a
  - *Oral: Surrogate: Levulinic acid (CAS #123-76-2)*: In the previously described GLP-compliant acute oral toxicity study conducted according to OECD Guideline 423/EU Method B.1 tris, female Sprague Dawley rats were administered single oral doses of 300 or 2,000 mg/kg (n= 3/dose) levulinic acid (purity unspecified) in water and observed up to 14 days. All high dose animals were found dead on day 2, and on the day of dosing clinical signs were reported including hunched posture, piloerection, and decreased activity. Due to loss of the animals in the high dose group, following the same study design, a second 300 mg/kg treatment group was treated and observed. At 300 mg/kg, no mortalities and no treatment related effects were reported on clinical signs of toxicity. No abnormal findings were reported at necropsy for both the 300 mg/kg and 2,000 mg/kg treatment groups (Klimisch 1, reliable without restriction) (Unnamed study 2016, 001 Key, ECHA, CAS #19856-23-6, 2023).
    - *Clinical signs of piloerection and decreased activity were identified at lethal doses; however, these clinical signs were not reported for the non-lethal dose of 300 mg/kg/day.*
  - *Dermal: Surrogate: Levulinic acid (CAS #123-76-2)*: In the previously described GLP-compliant dermal acute toxicity study conducted according to OECD Guideline 402/EU Method B.3/EPA OPPTS 870.1200, male and female Sprague Dawley rats (5/sex) were administered topical applications of 2,000 mg/kg undiluted levulinic acid (purity unspecified) for 24 hours under semiocclusive conditions and followed by a 14-day observation period. No treatment related effects on mortality, clinical signs of toxicity, or gross pathology at necropsy were reported (Klimisch 1, reliable without restriction) (Unnamed study 2017, 001 Key, ECHA, CAS #19856-23-6, 2023).

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): L
Sodium levulinate was assigned a score of Low for neurotoxicity (repeated dose) based on the lack of neurotoxicity observed at oral doses up to 1,000 mg/kg/day, the highest dose tested, in an OECD Guideline 408 repeated dose toxicity in rats exposed to the surrogate levulinic acid. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when LOAEL values are greater than 300 mg/kg/day for 30-day studies (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data including neurological examination for a strong surrogate.

- 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 2023a,c
  - *Oral: Surrogate: Levulinic acid (CAS #123-76-2)*: In the previously described GLP-compliant repeated dose toxicity study conducted according to OECD Guideline 408/EU Method B.26, male and female Wistar rats (10/sex/basic group, 6/sex/satellite group) were administered 0, 100, 500, and 1,000 mg/kg/day levulinic acid (purity not specified) in water via gavage, 7 days per week, for 90 days. No adverse treatment related effects were reported for clinical signs of neurotoxicity, neurobehavioral examination, and absolute and relative organ weights, gross pathology, and histopathology of the brain and spinal cord. Incidental and sporadic findings were either found in controls, did not show dose-dependence, are common to the age, sex, and species of the test animals, and/or were inconsistent without findings in relating parameters (Klimisch 1, reliable without restriction) (Unnamed study 2021, Key, ECHA, CAS #19856-23-6, 2023). *ToxServices established a neurotoxicity NOAEL of 1,000 mg/kg/day, the highest dose tested, based on no adverse neurological effects observed.*

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

Sodium levulinate was assigned a score of Moderate for skin sensitization based on ToxServices classifying it as a Category 1B skin sensitizer based on positive results in an *in vitro* OECD Guideline 442E study with the target chemical, positive results in an LLNA (BrdU-ELISA) with the strong surrogate levulinic acid, and self-classifications as a Category 1 skin sensitizer by the majority of ECHA notifiers. GreenScreen® criteria classify chemicals as a Moderate hazard for skin sensitization when they are classified to Category 1B (CPA 2018b). The confidence in the score is low due to mixed results in the OECD 442 assays and because the sub-classification criteria for the LLNA (BrdU-ELISA) are not yet agreed upon.

- 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 2023a
  - *In vitro*: In a GLP-compliant *in vitro* skin sensitization human cell line activation test (h-CLAT) conducted according to OECD Guideline 442E, THP-1, monocytic leukemia cells, were exposed to concentrations of 39.1 to 5,000 µg/mL sodium levulinate (purity not specified) for 24 hours, performed in duplicate. Following exposure, the expression of CD86 and CD54, cell surface antigens, were measured by flow cytometry. The vehicle (Roswell Park Memorial Institute (RPMI) media), negative (lactic acid (LA)), and positive controls (2,4-dinitrochlorobenzene (DNCB) and nickel sulfate (NiSO4)) were valid. The relative fluorescence intensity (RFI) values for CD86 were ≥ 150 at 5,000 µg/mL in experiment 2 only, and RFI values for CD54 were ≥ 200 at and above 2,500 µg/mL in experiment 1, and at 5,000 µg/mL in experiment 2. Cell viability was > 50% for all vehicle, negative, positive, and treatment groups. Based on these results, sodium levulinate has sensitizing potential (CD86 RFI ≥150 and CD54 ≥ 200 when cell viability is > 50%) (Klimisch 1, reliable without restriction) (Unnamed Study 2017, 003 WOE, ECHA, CAS #19856-23-6, 2023).
  - *In chemico*: In a GLP-compliant *in chemico* skin sensitisation: Direct Peptide Reactivity Assay (DPRA) conducted according to OECD Guideline 442C/Protocol No. 154 from ECVAM DB ALM, the interaction between sodium levulinate (purity not specified) and lysine and cysteine peptides were evaluated with high performance liquid chromatography

(HPLC), after exposure for 24 hours. The vehicle, negative, and positive controls (cinnamaldehyde with cysteine and lysine) were valid. The mean depletion of lysine and cysteine was 1.09% and 1.35%, respectively, with a mean depletion of 1.22%. Based on this negative prediction (mean % depletion of < 4.9%), the study authors concluded sodium levulinate presents no to minimal reactivity (Klimisch 1, reliable without restriction) (Unnamed Study 2018, 004 WOE, ECHA, CAS #19856-23-6, 2023).

- *In vitro*: In a GLP-compliant *in vitro* skin sensitization ARE-Nrf2 luciferase test conducted according to OECD Guideline 442D/EU Method B.60, genetically modified keratinocytes "Lu-Sens" were exposed up to 2,000 µg/mL sodium levulinate (purity not specified) for 48 hours, performed in triplicate in two experiments, and followed by MTT assay and evaluation of luciferase induction. The vehicle (DMEM and medium), negative (LA), and positive controls (p-phenylenediamine) were valid. Cell viability was ≥ 96% in the first experiment and ≥ 97% in the second experiment for all groups, and No substantial or reproducible dose-dependent increase in luciferase induction at or above 1.5 fold was observed in both experiments up to the maximum concentration tested. Based on these results, the study authors concluded sodium levulinate lacks the potential to activate the Nrf2 transcription factor and therefore lacks sensitizing potential (Klimisch 1, reliable without restriction) (Unnamed Study 2019, 005 WOE, ECHA, CAS #19856-23-6, 2023).
- *Surrogate: Levulinic acid (CAS #123-76-2)*: In a GLP-compliant local lymph node assay (LLNA) conducted according to OECD Guideline 442b (BrdU-ELISA), CBA/JN mice received topical applications of 5, 10, or 25% levulinic acid in olive oil:acetone (4:1), followed after one day by injection of 5-bromo-2'-deoxyuridine (BrdU) and after another day by sacrifice and evaluation of lymph nodes. There was a dose-related increase in proliferation, with stimulation indices (SI) of 1.31, 1.88, and 2.05 at the 5, 10, and 25% concentrations, respectively. Based on the SI values, the EC1.6 falls between 5% and 10%.
  - *There are currently no agreed upon subcategorization criteria for the LLNA* (BrdU-ELISA). A cut-off of ≤ 6% for 1A and > 6% for 1B has been proposed and validated (UN 2023).
- The majority of notifiers to ECHA (87/118) classified sodium levulinate as a Category 1 skin sensitizer (C&L Inventory, ECHA, CAS #19856-23-6, 2023).

- CIR 2021
  - Sodium levulinate was non-sensitizing in two separate HRIPTs (n=103 and n=53) at 0.4011% and 0.57% in formulated products.
- Based on these results, a score of Moderate was assigned. Sodium levulinate had negative predictions/results for 2 of out the 3 key events (key 1: peptide/protein binding, key 2: keratinocyte response, and key 3: dendritic cell response) in the adverse outcome pathway (AOP) for skin sensitization evaluated in the *in silico* OECD Guideline 442C DPRA, *in vitro* OECD Guideline 442D ARE-Nrf2 luciferase test, and *in vitro* OECD Guideline 442E h-CLAT skin sensitization studies, respectively. However, positive results were found in the OECD Guideline 442E h-CLAT study, indicating sodium levulinate has the potential to induce sensitization during key event 3, and the majority of notifiers to ECHA classified sodium levulinate as a skin sensitizer. In addition, the strong surrogate levulinic acid was positive in an LLNA (BrdU-ELISA), with an EC3 between 5% and 10%. Because the SI were 1.31 at 5% and 1.88 at 10%, the EC1.6 is most likely greater than 6%, the proposed cut-off for sub-classification. Therefore, ToxServices classified sodium levulinate as a Category 1B skin sensitizer, but confidence is reduced due to mixed results in the OECD 442 assays and because the sub-classification criteria for the LLNA (BrdU-ELISA) are not yet agreed upon.

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): DG
Sodium levulinate was assigned a score of Data Gap for respiratory sensitization based on a lack of adequate 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.
- OECD 2023
  - Sodium levulinate does not contain any structural alerts for respiratory sensitization (Appendix G).
- No data were identified for the target compound for this endpoint. Therefore, ToxServices attempted to evaluate the respiratory sensitization potential of sodium levulinate according to ECHA's guideline (ECHA 2017), which states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be 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 2017). Sodium levulinate does not contain any structural alerts, but is a potential skin sensitizer based on positive *in vitro* and *in vivo* data. According to the ECHA guidance, the positive skin sensitization results in animals and lack of structural alerts and evidence of respiratory sensitization indicate that there is insufficient positive data for the chemical to be classified as a respiratory sensitizer. However, the guidance requires negative skin sensitization data in order to conclude that the chemical is not a respiratory sensitizer. GreenScreen® criteria require negative data in order to assign a Low (i.e., a lack of alerts is not sufficient). Due to the positive data for skin sensitization and uncertainty regarding whether the mechanisms of sensitization could correspond to respiratory sensitization, a Data Gap was assigned.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): *L*
Sodium levulinate was assigned a score of Low based on negative results in an *in vitro* OECD Guideline 439 test for sodium levulinate with negative results in two HRIPTs for up to 0.57% target chemical. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is reduced as the OECD Guideline 439 does not differentiate between Category 3 and GHS Not classified, and the HRIPTs tested only very low concentrations.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- CIR 2021, ECHA 2023a
  - *In vitro:* <u>*Surrogate: Levulinic acid (CAS #123-76-2)*</u>: A GLP- compliant *in vitro* skin corrosion study conducted according to OECD Guideline 439 was performed in triplicate with the EpiSkin™ reconstructed human epidermis (RhE) model exposed to 20 µL undiluted levulinic acid (purity unspecified) for approximately 15 minutes with an incubation period of approximately 42 hours. The positive and negative controls were valid. The relative mean tissue viability at 15 minutes was 62% (which is > 50%); therefore, classification is not warranted. The study authors concluded the test substance was not irritating to the skin (Klimisch 1, reliable without restriction) (Unnamed Study 2016, 001 Key, ECHA, CAS #19856-23-6, 2023).

- CIR 2021
    - Sodium levulinate was non-irritating in two separate HRIPTs (n=103 and n=53) at 0.4011% and 0.57% in formulated products.
    - *Surrogate: Levulinic acid (CAS #123-76-2)*: A single 48-hour exposure to 4% levulinic acid in petrolatum via occlusive patch was non-irritating to the skin of human volunteers.
    - *Surrogate: Levulinic acid (CAS #123-76-2)*: In a study performed in 1979, however, undiluted levulinic acid was moderately to severely irritating when applied for 24 hours under occlusion to intact or abraded rabbit skin.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): vH
Sodium levulinate was assigned a score of Very High based on ToxServices classifying it as a GHS Category 1 eye irritant (positive results in an *in vitro* OECD Guideline 492 test and an OECD Guideline 437/EU Method B.47 bovine corneal opacity and permeability (BCOP) assay with the surrogate levulinic acid). GreenScreen® criteria classify chemicals as a Very High when they are classified to GHS Category 1 for skin or eye irritation (CPA 2018b). Confidence is high based on high quality, reliable data for a strong surrogate.

- 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 2023a,c
    - *In vitro: Surrogate: Levulinic acid (CAS #123-76-2)*: A GLP-compliant *in vitro* reconstructed human cornea-like epithelium (RhCE) test conducted according to OECD Guideline 492 was performed with the EPIOcular™ reconstructed human corneal model exposed to 50 µL undiluted levulinic acid (purity not specified) for approximately 30 minutes and examined 2 hours post-exposure. Positive (methyl acetate) and negative controls were valid. The relative mean tissue viability at 30 minutes was 2.5%. The study authors concluded the test substance was irritating to the eyes inducing serious eye damage. (Klimisch 2, reliable with restrictions) (Unnamed Study 2016, 001 Key, ECHA, CAS #19856-23-6, 2023).
        - *Based on the results of this study, classification as a Category 1 or Category 2 skin irritant is warranted; however, this study alone is not sufficient to assign Category 1 or 2.*
    - *In vitro: Surrogate: Levulinic acid (CAS #123-76-2)*: A GLP-compliant *in vitro* BCOP assay conducted according to OECD Guideline 437/EU Method B.47/OECD Guideline 160 was performed cattle corneas exposed to undiluted levulinic acid (purity not specified) for 10 minutes and examined 2 hours post-exposure. Positive and negative controls were valid. The *in vitro* irritation score (IVIS) was 84.29% (RSD – 12.68%), which is within the range of classification as a Category 1 eye irritant (> 55%); therefore, the study authors concluded the test substance induces serious eye damage (Klimisch 1, reliable without restriction) (Unnamed Study 2017, 002 Key, ECHA, CAS #19856-23-6, 2023).
    - Based on the combined results of the studies above, the authors of the ECHA dossier classified sodium levulinate as a Category 1 eye irritant.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): L
Sodium levulinate was assigned a score of Low for acute aquatic toxicity based on measured E(L)C_{50} of > 100 mg/L for all three trophic levels, fish, invertebrates, and algae. GreenScreen® criteria classify

chemicals as a Low hazard for acute aquatic toxicity when acute aquatic toxicity values are greater than 100 mg/L (CPA 2018b). The confidence in the score is high based on reliable, guideline studies for the target chemical and a strong surrogate 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 2023a
  - 96-hour LC_{50} (*Danio rerio*, zebrafish) > 100 mg/L (test. mat.) (Tradename: Hamilton-Buchana, GLP-compliant, OECD Guideline 203/EU Method C.1) (Klimisch 1, reliable without restriction) (Unnamed study 2018, 001 Key, ECHA, CAS #19856-23-6, 2023).
  - <u>*Surrogate: Levulinic acid (CAS #123-76-2)*</u>: 48-hour mobility EC_{50} (*Daphnia magna*) > 100 mg/L (nominal, test mat.) (GLP-compliant, OECD Guideline 202/EU Method C.2) (Klimisch 1, reliable without restriction) (Unnamed study 2021, 001 Key, ECHA, CAS #19856-23-6, 2023).
  - <u>*Surrogate: Levulinic acid (CAS #123-76-2)*</u>: 72-hour growth rate EC_{50} (*Desmodesmus subspicatus*, green algae) > 100 mg/L (nominal, test mat.) (GLP-compliant, OECD Guideline 201/EU Method C.3) (Klimisch 1, reliable without restriction) (Unnamed study 2021, 001 Key, ECHA, CAS #19856-23-6, 2023).

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

Sodium levulinate was assigned a score of Low for chronic aquatic toxicity based on measured a NOEC of > 100 mg/L for algae and modeled chronic values (ChVs) of 15,700 mg/L and 4,740 mg/L for the fish and invertebrate trophic levels, respectively. GreenScreen® criteria classify chemicals as a Low hazard for chronic aquatic toxicity when chronic aquatic toxicity values are greater than 10 mg/L (CPA 2018b). The confidence in the score is reduced due to the lack of measured data for the fish and aquatic invertebrate 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 2023a
  - <u>*Surrogate: Levulinic acid (CAS #123-76-2)*</u>: 72-hour growth rate EC_{10} and NOEC (*Desmodesmus subspicatus*, green algae) > 100 mg/L (nominal, test mat.) (GLP-compliant, OECD Guideline 201/EU Method C.3) (Klimisch 1, reliable without restriction) (Unnamed study 2021, 001 Key, ECHA, CAS #19856-23-6, 2023).
- U.S. EPA 2017a
  - Sodium levulinate belongs to the Neutral Organics ECOSAR chemical class. The most conservative predicted ChVs are 15,700 mg/L in fish, 4,740 mg/L in daphnia, and 3,990 mg/L in green algae (see Appendix H).

<u>Environmental Fate (Fate)</u>

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

Sodium levulinate was assigned a score of Very Low for persistence based on positive results in an OECD Guideline 301F ready biodegradability guideline studies for the surrogate levulinic acid (meeting the 10-day window). GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when soil, sediment, or water is the dominant environmental compartment and the 10-day window is

met (CPA 2018b). Confidence is high based on the high quality, measured biodegradation data for a strong surrogate.

- 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 2023a,d
  - *Surrogate: Levulinic acid (CAS #123-76-2)*: A non-GLP-compliant ready biodegradability: manometric respiratory test, conducted according to OECD Guideline 301F, was performed with activated sludge (adaptation specified) exposed to levulinic acid (purity not specified) at 100 mg/L for 28 days. At the end of the exposure period, the level of degradation was > 60% in 28 days, meeting the 10-day window. The study authors concluded levulinic acid is readily biodegradable (Klimisch 1, reliable with restrictions) (Unnamed study 2007, 001 Key, ECHA, CAS #123-76-2, 2023).
- U.S. EPA 2017b
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that sodium levulinate is expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 66.5% will partition to soil with a half-life of 17.33 days, 33.5% will partition to water with a half-life of 8.67 days, 0.0592% will partition to sediment with a half-life of 77.92 days, and 0.00111 will partition to air with a half-life of 5.13 days (Appendix I).

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL

Sodium levulinate was assigned a score of Very Low for bioaccumulation based on a low measured log K_{ow} of -0.62 and low estimated BCFs of -0.045 – 3.162. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when log K_{ow} values are no greater than 4 and BCF values are no greater than 100 (CPA 2018b). Confidence in the score is high as it is based on an experimental log K_{ow}, with support from bioaccumulation modeling data 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 2023a
  - Sodium levulinate has a measured log K_{ow} of -0.616 from an OECD Guideline 107/EU Method A.8 test (Klimisch 1, reliable without restriction) (Unname study 2018, 001 Key – Partition Coefficient, ECHA, CAS #19856-23-6, 2023).
- U.S. EPA 2017b
  - BCFBAF predicts a BCF of 3.162 using the regression based model based on a measured log K_{ow} of -0.62, and a BCF of -0.045 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix I).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*

Sodium levulinate was assigned a score of Low for reactivity based on ToxServices not classifying it as a reactive chemical under GHS criteria according to its chemical structure. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when no GHS classification is available (CPA 2018b). The confidence in the score was reduced as it was not based on an authoritative list or 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.

- ECHA 2023a
    - The authors of the ECHA dossier reported that sodium levulinate was non-explosive and non-oxidizing due to a lack of chemical groups associated with explosive and oxidizing properties, and non-oxidizing on the basis of its chemical structure. Additionally, no evidence of pyrophoric properties was identified by the authors of the ECHA dossier.
    - In the previously described EU Method A.16 relative self-ignition temperature for solids test, no self-ignition of sodium levulinate was observed up to the melting point of 170.2°C (Klimisch 1, reliable without restriction) (Unnamed study 2017, 001 Key – Auto flammability, ECHA, CAS #19856-23-6, 2023a).
- BeanTown Chemical 2021
    - A material safety data sheet for sodium levulinate (purity unspecified) reports that it has an instability rating of 0 from the National Fire Protection Association (NFPA) ("Normally stable, even under fire exposure conditions, and is not reactive with water").
- Based on the weight of evidence, ToxServices did not identify sodium levulinate as reactive. It was not ignitable, and it is not expected to be explosive or self-reactive based on chemical structure and an NFPA instability rating of 0. Sodium levulinate has no reactive functional groups that would make it oxidizing or explosive, and it is not a peroxide. As it is not explosive, it does not require desensitization. Overall, sodium levulinate is not classified for any of the reactivity sub endpoints under GHS (UN 2023). No data were found regarding corrosivity to metal.

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

Sodium levulinate was assigned a score of Low for flammability based on ToxServices not classifying it as a flammable solid under GHS criteria based on results of flammability tests. GreenScreen® criteria classify chemicals as a Low hazard for flammability when no GHS classification is available (CPA 2018b). The confidence in the score is high based on high quality, measured data on flammability 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 2023a
    - In a GLP-compliant preliminary screening test for flammable solid conducted according to UN Manual of Tests and Criteria: Test N.1/EU Method A.10, sodium levulinate melted to a yellow-brown liquid and boiled in contact with flame, and although the substance got darker, no signs of flammability were observed. The study authors concluded sodium levulinate is a non-flammable solid, and does not warrant classification (Klimisch 1, reliable without restriction) (Unnamed study 2016, 001 Key – Flammability, ECHA, CAS #19856-23-6, 2023a).
    - In the previously described EU Method A.16 relative self-ignition temperature for solids test, no self-ignition of sodium levulinate was observed up to the melting point of 170.2°C (Klimisch 1, reliable without restriction) (Unnamed study 2017, 001 Key – Auto flammability, ECHA, CAS #19856-23-6, 2023a).
- BeanTown Chemical 2021
    - A material safety data sheet for sodium levulinate (purity unspecified) reported a flammability rating of 0 from NFPA ("Material that in itself is normally stable, even under fire exposure conditions, and is not reactive with water. Example, liquid nitrogen.").
- Based on the above data, ToxServices did not classify sodium levulinate as a flammable solid under GHS criteria (UN 2023).

Use of New Approach Methodologies (NAMs)[9] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for endocrine activity, respiratory sensitization, chronic aquatic toxicity, persistence, and bioaccumulation, and a variety of *in vitro* studies for genotoxicity, skin sensitization, skin irritation, and eye irritation. 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 2020). 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 2020):

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

As shown in Table 3, Type I (input data) uncertainties in sodium levulinate's NAMs dataset include limited experimental data for carcinogenicity, endocrine activity, skin and respiratory sensitization, and skin irritation, and lack of established test methods for respiratory sensitization. Sodium levulinate's Type II (extrapolation output) uncertainties include lack of defined applicability domains of OECD QSAR Toolbox and ToxCast models in examination of structural alerts, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions, the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization, and inability of individual *in vitro* skin sensitization, skin irritation and eye irritation tests to completely differentiate certain GHS categories. Some of sodium levulinate's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 3: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: Only limited experimental data are available. **Endocrine:** Insufficient *in vivo* data for hormone signaling pathways are available. **Respiratory sensitization**: No experimental data are available and there are no validated test methods. **Skin irritation**: Only one *in vitro* study available (OECD 439)
Type II Uncertainty: Extrapolation Output	**Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in

[9] 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[10]. 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).[11] The *in vitro* chromosome aberration assay (OECD Guideline 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[12]. **Endocrine activity:** ToxCast models don't define applicability domain, and *in silico* modeling of receptor binding is unknown due to lack of consideration of metabolism and other toxicokinetic factors. **Skin sensitization:** The *in chemico* and *in vitro* assays evaluating key events in the skin sensitization adverse outcome pathway (AOP) don't typically include metabolism or abiotic transformation to address chemicals that are pro-haptens or pre-haptens, respectively. Further, each test has their applicable domain such as limitations in test substance solubility or log $K_{ow.}$[13] **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization. **Skin irritation**: The OECD Guideline 439 test is only used to identify irritating substances (GHS Category 2) and non-irritating substances (no category), and does not allow the classification as a mild skin irritant (GHS Category 3)[14]. **Eye irritation**: The BCOP (OECD Guideline 437) test is not recommended for identifying GHS Category 2A or 2B irritants[15]. The RhCE test (OECD Guideline 492) cannot differentiate between Category 2 and Category 1, or between Category 2A and Category 2B. There is no single *in vitro* method that can replace an *in vivo* eye irritation test[16]. Therefore, this method is not recommended for

[10] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[11] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[12] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352
[13] 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
[14] https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1614097324&id=id&accname=guest&checksum=D664A7EDCDE297919BE9A478941EBEC6
[15] https://www.oecd-ilibrary.org/docserver/9789264203846-en.pdf?expires=1614095760&id=id&accname=guest&checksum=1613168F64BDB3558225572BDD75FC8D
[16] https://www.oecd.org/env/ehs/testing/E492_2017.pdf

	identifying eye irritants (Category 2) or substances causing serious eye damage (Category 1) (ECHA 2017).	
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* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In silico* modeling: ToxCast models/ Danish QSAR
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	Y	*In vitro* and *in chemico* tests: OECD Guideline 442C, D, and E
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	Y	*In vitro* testing: OECD Guideline 439 RhE test
Eye irritation	Y	*In vitro* testing: OECD Guideline 492 reconstructed human cornea-like epithelium test (EpiOcular™) / OECD Guideline 437 bovine corneal opacity and permeability (BCOP) test
Acute aquatic toxicity	N	
Chronic aquatic toxicity	Y	*In silico* modeling: ECOSAR
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: OECD 301C, F Biodegradation tests
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

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United States Environmental Protection Agency (U.S. EPA). 2023a. Safer Chemical Ingredients List (SCIL). Available: https://www.epa.gov/saferchoice/safer-ingredients

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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 Sodium Levulinate (CAS #19856-23-6)

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	Sodium levulinate	19856-23-6	*L*	L	*L*	L	DG	M	L	L	*L*	L	*M*	DG	*L*	vH	L	*L*	vL	vL	*L*	L

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Sodium levulinate	2

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

Table 6

Chemical Name	Final GreenScreen® Benchmark Score
Sodium levulinate	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												

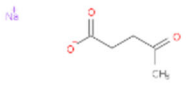
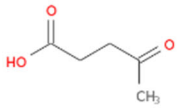
APPENDIX C: ChemMine Similarity Output for Sodium Levulinate (CAS #19856-23-6)

Compound Similarity

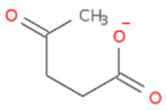
3 compound(s) in workbench

Select two compounds to compare from the grid below.

Selected Compounds

Target: Sodium levulinate (CAS #19856-23-6)	Surrogate: Levulinic acid (CAS #123-76-2)
	
remove	remove

AP Tanimoto: 1
MCS Tanimoto: 0.8889
MCS Size: 8
MCS Min: 1.0000
MCS Max: 0.8889
SMILES: C(=O)(CCC(=O)[O-])C Target: Sodium levulinate (CAS #19856-23-6)

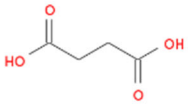
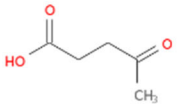


Compound Similarity

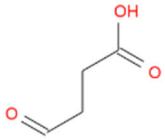
3 compound(s) in workbench

Select two compounds to compare from the grid below.

Selected Compounds

Surrogate: Succinic acid (CAS #110-15-6)	Surrogate: Levulinic acid (CAS #123-76-2)
	
remove	remove

AP Tanimoto: 0.6
MCS Tanimoto: 0.7778
MCS Size: 7
MCS Min: 0.8750
MCS Max: 0.8750
SMILES: C(=O)CCC(=O)O Surrogate: Succinic acid (CAS #110-15-6)

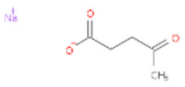
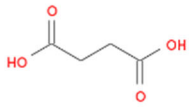


Compound Similarity

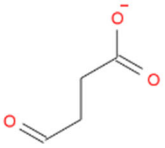
3 compound(s) in workbench

Select two compounds to compare from the grid below.

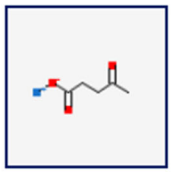
Selected Compounds

Target: Sodium levulinate (CAS #19856-23-6)	Surrogate: Succinic acid (CAS #110-15-6)
	
remove	remove

AP Tanimoto: 0.6
MCS Tanimoto: 0.7000
MCS Size: 7
MCS Min: 0.8750
MCS Max: 0.7778
SMILES: C(=O)CCC(=O)[O-] Target: Sodium levulinate (CAS #19856-23-6)



APPENDIX D: Pharos Output for Sodium Levulinate (CAS #19856-23-6)



19856-23-6

SODIUM LEVULINATE

ALSO CALLED 243-378-4, Levulinic acid sodium salt, Pentanoic acid, 4-oxo-, sodium salt, Pentanoic acid, 4-oxo-,s...

View all synonyms (9)

Share Profile

Hazards | Properties | Functional Uses | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

		Group I Human					Group II and II* Human								Ecotox			Fate		Phys
	GREENSCREEN®	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx
List Hazard Summary	LT-UNK	-	-	-	-	-	pC	pC	-	-	-	pC	-	pC	pC	-	-	-	-	-	-

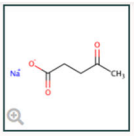
Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GREENSCREEN®	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Acute Mammalian Toxicity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H302 - Harmful if swallowed (unverified) [Acute toxicity (oral) - Category 4]	

Systemic Toxicity/Organ Effects-Single Exposure	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
Skin Sensitization	pC	NoGS	EU - Manufacturer REACH hazard submissions	H317 - May cause an allergic skin reaction (unverified) [Skin sensitization - Category 1]	
Skin Irritation/Corrosivity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	
Eye Irritation/Corrosivity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H318 - Causes serious eye damage (unverified) [Serious eye damage/eye irritation - Category 1]	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H319 - Causes serious eye irritation (unverified) [Serious eye damage/eye irritation - Category 2A]	
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	

APPENDIX E: ToxCast Modeling Results for Sodium Levulinate (CAS #19856-23-6)



Sodium levulinate

19856-23-6 | DTXSID60173608

Searched by DTXSID60173608

Bioactivity - ToxCast: Models

EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
COMPARA (Consensus)	Androgen	0.00	0.00	0
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0

APPENDIX F: Danish QSAR Endocrine Results for Sodium Levulinate (CAS #19856-23-6)

Endocrine and Molecular Endpoints

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Estrogen Receptor α Activation (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Sodium/iodide symporter (NIS), higher sensitivity		N/A	N/A	NEG_IN	N/A
Sodium/iodide symporter (NIS), higher specificity		N/A	N/A	NEG_IN	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L				1299.712	

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
- μM				11192.84	
- Positive for IC_{50} ≤ 10 μM					
- Positive for IC_{50} ≤ 100 μM					
- Domain				OUT	
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L				414.9798	
- μM				3573.715	
- Positive for IC_{50} ≤ 10 μM					
- Positive for IC_{50} ≤ 100 μM					
- Domain				OUT	
Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) Inhibition at max. 10 μM (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A

Retinoic Acid Receptor (RAR) inhibition at max. 10 µM (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Binding (Human *in vitro*)	N/A	NEG_IN	POS_OUT	NEG_IN	NEG_IN
Pregnane X Receptor (PXR) Binding (Human *in vitro*) NEW	NEG	N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Rat *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
CYP3A4 Induction (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
	Exp	Battery	CASE Ultra	Leadscope	SciQSAR

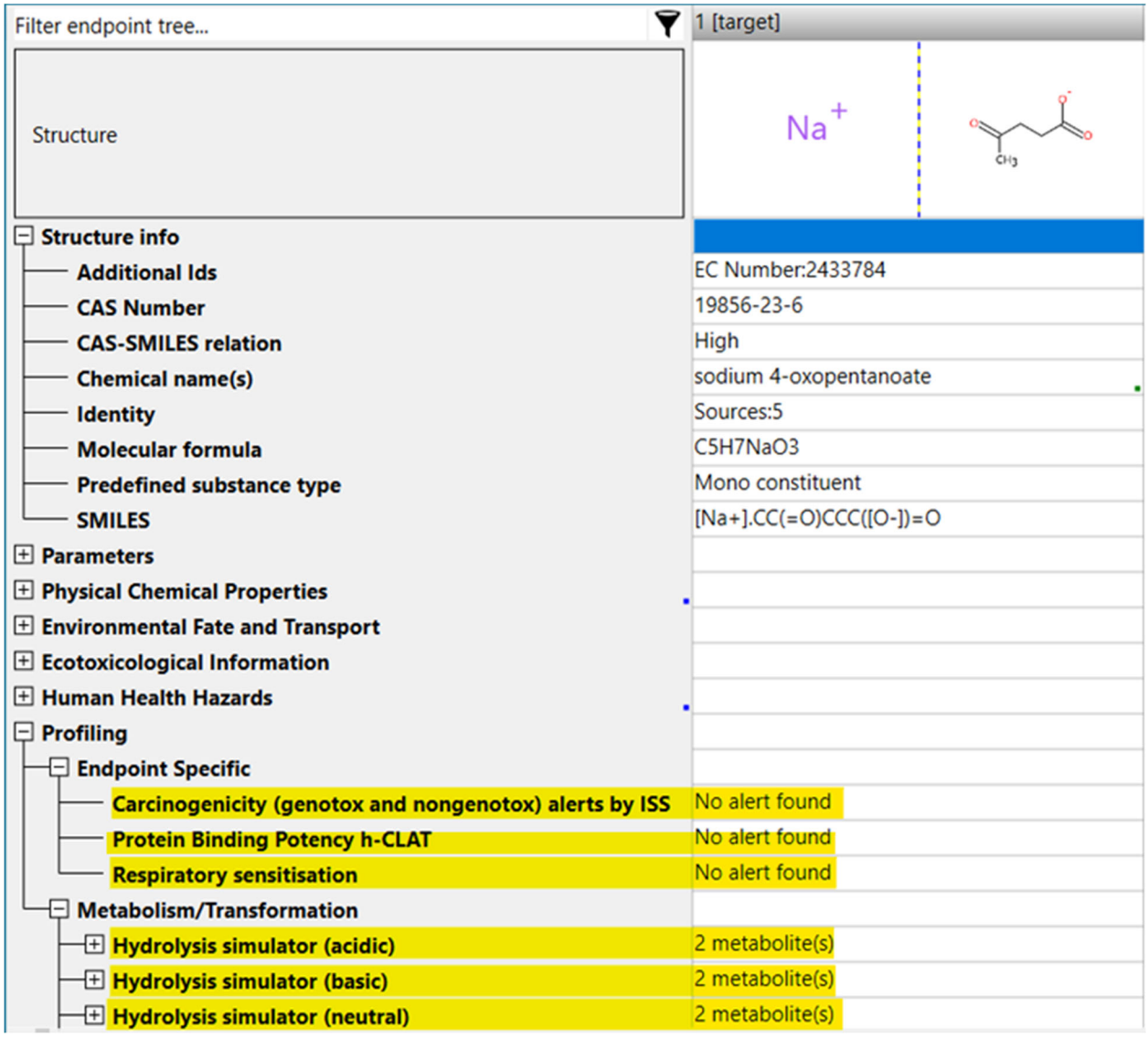
DTU-developed models

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, non cyclic structure
- metabolites from *in vivo* Rat metabolism simulator only	Non binder, non cyclic structure
- metabolites from Rat liver S9 metabolism simulator only	Non binder, non cyclic structure
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found

OECD QSAR Toolbox v.4.2 profilers

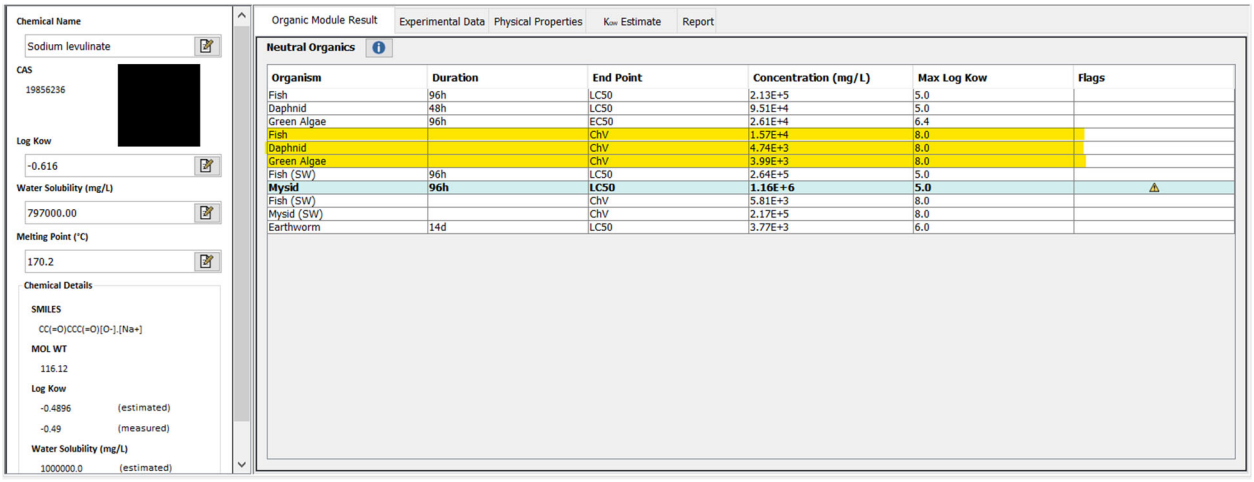
Profiler predictions are supporting information to be used together with the relevant QSAR predictions

APPENDIX G: OECD Toolbox Profiling Results for Sodium Levulinate (CAS #19856-23-6)



Filter endpoint tree...	1 [target]
Structure	
Structure info	
Additional Ids	EC Number:2433784
CAS Number	19856-23-6
CAS-SMILES relation	High
Chemical name(s)	sodium 4-oxopentanoate
Identity	Sources:5
Molecular formula	C5H7NaO3
Predefined substance type	Mono constituent
SMILES	[Na+].CC(=O)CCC([O-])=O
Parameters	
Physical Chemical Properties	
Environmental Fate and Transport	
Ecotoxicological Information	
Human Health Hazards	
Profiling	
Endpoint Specific	
Carcinogenicity (genotox and nongenotox) alerts by ISS	No alert found
Protein Binding Potency h-CLAT	No alert found
Respiratory sensitisation	No alert found
Metabolism/Transformation	
Hydrolysis simulator (acidic)	2 metabolite(s)
Hydrolysis simulator (basic)	2 metabolite(s)
Hydrolysis simulator (neutral)	2 metabolite(s)

APPENDIX H: ECOSAR Modeling Results for Sodium Levulinate (CAS #19856-23-6)



Chemical Name
Sodium levulinate

CAS
19856236 [redacted]

Log Kow
-0.616

Water Solubility (mg/L)
797000.00

Melting Point (°C)
170.2

Chemical Details

SMILES
CC(=O)CCC(=O)[O-].[Na+]

MOL WT
116.12

Log Kow
-0.4896 (estimated)
-0.49 (measured)

Water Solubility (mg/L)
1000000.0 (estimated)

Organic Module Result | Experimental Data | Physical Properties | Kow Estimate | Report

Neutral Organics

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	2.13E+5	5.0	
Daphnid	48h	LC50	9.51E+4	5.0	
Green Algae	96h	EC50	2.61E+4	6.4	
Fish		ChV	1.57E+4	8.0	
Daphnid		ChV	4.74E+3	8.0	
Green Algae		ChV	3.99E+3	8.0	
Fish (SW)	96h	LC50	2.64E+5	5.0	
Mysid	**96h**	**LC50**	**1.16E+6**	**5.0**	⚠
Fish (SW)		ChV	5.81E+3	8.0	
Mysid (SW)		ChV	2.17E+5	8.0	
Earthworm	14d	LC50	3.77E+3	6.0	

APPENDIX I: EPI Suite™ Modeling Results for Sodium Levulinate (CAS #19856-23-6)

CAS Number: 19856-23-6
SMILES : CC(=O)CCC(=O)O([Na])
CHEM : Pentanoic acid, 4-oxo-, sodium salt
MOL FOR: C5 H7 O3 Na1
MOL WT : 138.10
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): -0.62
Boiling Point (deg C) : ------
Melting Point (deg C) : 170.20
Vapor Pressure (mm Hg) : 0.000139
Water Solubility (mg/L): 7.97E+005
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = -4.30

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 449.82 (Adapted Stein & Brown method)
Melting Pt (deg C): 185.51 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 1.57E-008 (Modified Grain method)
VP (Pa, 25 deg C) : 2.09E-006 (Modified Grain method)
Subcooled liquid VP: 0.00379 mm Hg (-999 deg C, user-entered VP)
: 0.506 Pa (-999 deg C, user-entered VP)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 4.519e+004
log Kow used: -0.62 (user entered)
melt pt used: 170.20 deg C

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 4.2317e+005 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Neutral Organics-acid

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 4.91E-010 atm-m3/mole (4.98E-005 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 3.169E-011 atm-m3/mole (3.211E-006 Pa-m3/mole)
VP: 0.000139 mm Hg (source: User-Entered)
WS: 7.97E+005 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: -0.62 (user entered)
Log Kaw used: -7.697 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 7.081
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.7718
Biowin2 (Non-Linear Model) : 0.8249
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.2847 (days-weeks)
Biowin4 (Primary Survey Model) : 4.0436 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.8290
Biowin6 (MITI Non-Linear Model): 0.9374
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.6034
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): 0.505 Pa (0.00379 mm Hg)
Log Koa (Koawin est): 7.081
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 5.94E-006
Octanol/air (Koa) model: 2.96E-006
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.000214
Mackay model : 0.000475
Octanol/air (Koa) model: 0.000237

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 2.0928 E-12 cm3/molecule-sec
Half-Life = 5.111 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 61.330 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.000345 (Junge-Pankow, Mackay avg)
0.000237 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 1 L/kg (MCI method)
Log Koc: 0.000 (MCI method)

Koc : 1.025 L/kg (Kow method)
Log Koc: 0.011 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.9671 days (HL = 0.01079 days)
Log BCF Arnot-Gobas method (upper trophic) = -0.045 (BCF = 0.9008)
Log BAF Arnot-Gobas method (upper trophic) = -0.045 (BAF = 0.9008)
log Kow used: -0.62 (user entered)

Volatilization from Water:
Henry LC: 3.17E-011 atm-m3/mole (calculated from VP/WS)
Half-Life from Model River: 2.171E+007 hours (9.046E+005 days)
Half-Life from Model Lake : 2.368E+008 hours (9.869E+006 days)

Removal In Wastewater Treatment:
Total removal: 1.85 percent
Total biodegradation: 0.09 percent
Total sludge adsorption: 1.76 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**0.00111**	**123**	**1000**
Water	**33.5**	**208**	**1000**
Soil	**66.5**	**416**	**1000**
Sediment	**0.0592**	**1.87e+003**	**0**

Persistence Time: 391 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.00111	123	1000
Water	33.5	208	1000
water	(33.5)		
biota	(4.05e-007)		
suspended sediment	(5.02e-005)		
Soil	66.5	416	1000
Sediment	0.0592	1.87e+003	0

Persistence Time: 391 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)

Air	0.00112	123	1000
Water	34.4	208	1000
water	(34.4)		
biota	(4.16e-007)		
suspended sediment	(5.12e-006)		
Soil	65.6	416	1000
Sediment	0.0596	1.87e+003	0
Persistence Time: 388 hr			

APPENDIX J: Change in Benchmark Score

Table 4 provides a summary of changes to the GreenScreen® Benchmark™ for sodium levulinate. This is a new assessment.

Table 4: Change in GreenScreen® Benchmark™ for Sodium Levulinate

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
November 20, 2023	BM-2	v. 1.4	New assessment

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