# 4,5-DICHLORO-2-OCTYL-2H-ISOTHIAZOL-3-ONE

## (CAS #64359-81-5)

## GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

**Prepared by:**

**ToxServices LLC**

**Assessment Date: October 16, 2023**

**Expiration Date: October 16, 2028**

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# GreenScreen® Executive Summary for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)

4,5-Dichloro-2-octyl-2H-isothiazol-3-one is an isothiazolinone-based biocide that is used to treat wood and as a preservative and antifouling agent in paints, adhesives, coatings, fuels, metal working fluids, and resin emulsions, and in paints pulp/paper mills, cooling water systems, oil field operations, industrial process waters, and air washers systems. It is an off-white to beige solid that is slightly soluble in water. It is not a volatile organic compound (VOC) based on its low vapor pressure and because it boils prior to decomposition. 4,5-Dichloro-2-octyl-2H-isothiazol-3-one is not flammable or reactive.

4,5-Dichloro-2-octyl-2H-isothiazol-3-one 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 2e
  - Moderate Group I Human Health (endocrine activity – E)
- Benchmark 2f
  - Very High Group II Human Health (acute toxicity – AT, systemic toxicity (single dose) – STs, skin irritation – IrS, and eye irritation – IrE)
  - High Group II* Human Health (skin sensitization – SnS*)
  - Very High Ecotoxicity (acute aquatic toxicity – AA and chronic aquatic toxicity – CA)

A data gap (DG) exists for respiratory sensitization – SnR*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), 4,5-dichloro-2-octyl-2H-isothiazol-3-one meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if 4,5-dichloro-2-octyl-2H-isothiazol-3-one were assigned a High score for the data gap SnR*, it would still be categorized as a Benchmark 2 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* data for endocrine activity and genotoxicity, and *in silico* modeling for respiratory sensitization, bioaccumulation, and persistence. 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 4,5-dichloro-2-octyl-2H-isothiazol-3-one's NAMs dataset include a lack of sufficient experimental data for endocrine activity and respiratory sensitization, and a lack of validated test methods for respiratory sensitization. 4,5-Dichloro-2-octyl-2H-isothiazol-3-one's Type II (extrapolation output) uncertainties include the inability of *in vitro* genotoxicity assays to mimic *in vivo* conditions, uncertainty regarding the *in vivo* relevance of *in vitro* endocrine activity assays, and that OECD Toolbox only identifies structural alerts and ECHA's guidance on respiratory sensitization does not evaluate non-immunologic mechanisms.

**GreenScreen® Hazard Summary Table for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one**

| 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 | *M* | vH | *vH* | L | | L | H | DG | vH | vH | vH | vH | *L* | *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 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)

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

**GreenScreen® Assessment (v.1.4) Prepared By:**
Name: Jennifer Rutkiewicz, Ph.D.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: July 18, 2023

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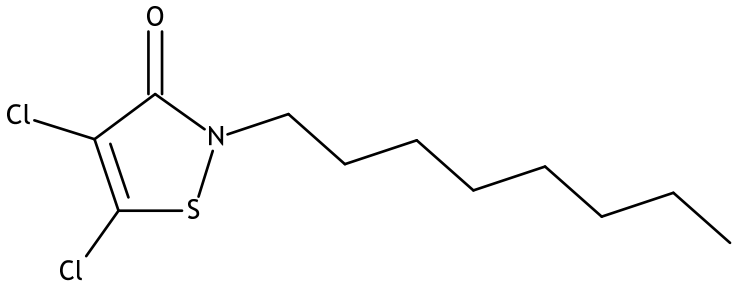
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: August 29, 2023; October 16, 2023

Expiration Date: October 16, 2028[2]

**Chemical Name:** 4,5-Dichloro-2-octyl-2H-isothiazol-3-one

**CAS Number:** 64359-81-5

**Chemical Structure(s):**

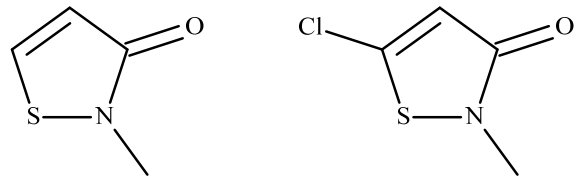



**Also called:** Kathon 930; 4,5-dichloro-2-(n-octyl)-3(2H)-isothiazolone; 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one; 4,5-Dichloro-2-octylisothiazol-3(2H)-one; 4,5-Dichloro-2-octyl-isothiazolone; 4,5-Dichloro-2-octyl-3(2H)-isothiazolone; 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone; 3(2H)-Isothiazolone, 4,5-dichloro-2-octyl-; 4,5-dichloro-2-octyl-1,2-thiazol-3-one; 4,5-dichloro-2-octyl-3-isothiazolone; 4,5-Dichloro-2-octylisothiazolone; 4,5-Dichloro-N-octyl-3(2H)-isothiazolone; 2-n-octyl-4,5-dichloro-1-isothiazolin-3-one; 4,5-dichloro-2-n-octyl-3-isothiazolone; Dichloro-2-n-octyl-3(2H)-isothiazolone; dichlorooctylisothiazolinone; 4,5-dichloro-2-(n-octyl)-3(2H)-isothiazolone; 4,5-dichloro-2-octyl-4-isothiazolin-3-one; 4,5-Dichloro-2h-octyl-3(2H)-isothiazolone; 4,5-dichloro-2-octyl-1,2-thiazol-3(2H)-one; 4,5-dichloro-2-octyl-isothiazolone; 4,5-dichloro-2-octyl-2,3-dihydro-1,2-thiazol-3-one; dichloro-2-n-oct yl-3 (2H) isothiazolone, 4,5- (PubChem 2023)

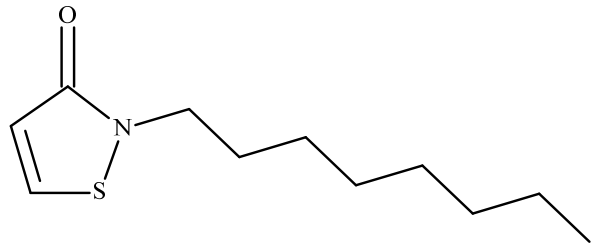
**Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):**
4,5-Dichloro-2-octyl-2H-isothiazol-3-one has a robust dataset; however, it lacks chronic data to evaluate carcinogenicity. Therefore, ToxServices used data on a mixture of 5-chloro-2-methyl-2H-isothiazolin-3-one (CMIT) and 2-methyl-2H-isothiazolin-3-one (MIT) (CAS # 55965-84-9) and 2-octyl-3(2H)-

[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).

isothiazolone (OIT)(CAS #26530-20-1), which are similar isothiazolinone-based compounds that were used as read-across chemicals in the evaluation of the proposed classification for 4,5-dichloro-2-octyl-2H-isothiazol-3-one in the European Union (EU) (ECHA 2018a,b,c), to evaluate this endpoint. These surrogates share the isothiazolinone moiety, which is expected to be the greatest contributor to toxicity, with the target compound. CMIT and MIT are smaller compounds than the target, as they have a methyl group rather than an 8-carbon chain, but like the target, one of the compounds is chlorinated. OIT differs from the target by the absence of the chlorine substituents.




Mixture of 5-chloro-2-methyl-2H-isothiazolin-3-one (CMIT) and 2-methyl-2H-isothiazolin-3-one (MIT)(CAS # 55965-84-9)




2-Octyl-3(2H)- isothiazolone (OIT)(CAS #26530-20-1)

**Identify Applications/Functional Uses:** (U.S. EPA 2020a, ECHA 2018a,b,c)
1. Wood treatment
2. Preservative
3. Antifouling agent

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

**GreenScreen® Summary Rating for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one**[3,4 5,6]**:** 4,5-Dichloro-2-octyl-2H-isothiazol-3-one 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 2e
    - Moderate Group I Human Health (endocrine activity – E)
- Benchmark 2f

[3] 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.
[4] See Appendix A for a glossary of hazard endpoint acronyms.
[5] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[6] 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.

- Very High Group II Human Health (acute toxicity – AT, systemic toxicity (single dose) – STs, skin irritation – IrS, and eye irritation – IrE)
- High Group II* Human Health (skin sensitization – SnS*)
- Very High Ecotoxicity (acute aquatic toxicity – AA and chronic aquatic toxicity – CA)

A data gap (DG) exists for respiratory sensitization – SnR*. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), 4,5-dichloro-2-octyl-2H-isothiazol-3-one meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if 4,5-dichloro-2-octyl-2H-isothiazol-3-one were assigned a High score for the data gap SnR*, it would still be categorized as a Benchmark 2 Chemical.

**Figure 1: GreenScreen® Hazard Summary Table for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one**

| 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** | *M* | **vH** | *vH* | **L** | | **L** | **H** | DG | **vH** | **vH** | **vH** | **vH** | *L* | *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

Hydrolysis, photolysis, and biodegradation studies on 4,5-dichloro-2-octyl-2H-isothiazol-3-one have reported a number of degradation products (described under the Persistence endpoint), as summarized in Table 1, below. As these products are not LT-1 chemicals, the overall Benchmark of the chemical is not impacted by its degradation products.

**Table 1: Environmental Transformation Product Summary**

| Life Cycle Stage | Transformation Pathway | Environmental Transformation Product | CAS # | Feasible (Yes or No) | Relevant (Yes or No) | GreenScreen® List Translator Score or GreenScreen® Benchmark™ Score[7,8] |
|---|---|---|---|---|---|---|
| End of life | Hydrolysis Biodegradation (estuarine water) | 2-Chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Hydrolysis Biodegradation (estuarine water) | 1-Chloro2-(n-octylcarbamoyl)-1-ethene sulfonic acid | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Hydrolysis | N-(n-Octyl) propiolic acid amide | N/A | Yes | Yes | Not present in Pharos database |

[7] The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to screen for GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2023) is an online list-searching tool that is used to screen chemicals against the lists in the List Translator electronically.
[8] A GreenScreen® assessment of a transformation product depends on the Benchmark score of the parent chemical (see GreenScreen® Guidance).

| Table 1: Environmental Transformation Product Summary | | | | | | |
|---|---|---|---|---|---|---|
| **Life Cycle Stage** | **Transformation Pathway** | **Environmental Transformation Product** | **CAS #** | **Feasible (Yes or No)** | **Relevant (Yes or No)** | **GreenScreen® List Translator Score or GreenScreen® Benchmark™ Score[7,8]** |
| End of life | Photolysis<br>Biodegradation (estuarine water) | N-(n-Octyl) oxamic acid (NNOOA) | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Biodegradation (freshwater-sediment)<br>Biodegradation (seawater-sediment) | N-(n-Octyl) malonamic acid (NNOMA) | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Biodegradation (freshwater-sediment)<br>Biodegradation (seawater-sediment) | N-(n-Octyl) acetamide | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Biodegradation (freshwater-sediment) | 3,3'Dithiobis-(n-octyl)-3-chloropropenamide | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Biodegradation (freshwater-sediment) | 2-Chloro-3-(formyldithio)-N-octylpropenamide | N/A | Yes | Yes | Not present in Pharos database |
| End of life | Hydrolysis | Sulfinic acid | 24053-94-9 | Yes | Yes | Not present in Pharos database |
| End of life | Hydrolysis | Propiolic acid amine | 471-25-0 | Yes | Yes | LT-U |

## Introduction

4,5-Dichloro-2-octyl-2H-isothiazol-3-one is an isothiazol-based biocide that is used to treat wood and as a preservative and antifouling agent in paints, adhesives, coatings, fuels, metal working fluids, and resin emulsions, and in paints pulp/paper mills, cooling water systems, oil field operations, industrial process waters, air washers systems (U.S. EPA 2020a, ECHA 2018a,b,c).

ToxServices assessed 4,5-dichloro-2-octyl-2H-isothiazol-3-one 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 2015a).

4,5-Dichloro-2-octyl-2H-isothiazol-3-one 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),[9] 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 4,5-dichloro-2-octyl-2H-isothiazol-3-one can be found in Appendix C.

- 4,5-Dichloro-2-octyl-2H-isothiazol-3-one is an LT-U chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- 4,5-Dichloro-2-octyl-2H-isothiazol-3-one is not listed on the U.S. DOT list.
- 4,5-Dichloro-2-octyl-2H-isothiazol-3-one is on the following lists for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.
    - EU - GHS (H-Statements) Annex 6 Table 3-1 H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]
    - GHS - New Zealand: Hazardous to the aquatic environment - chronic category 1
    - GHS – Australia: H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]
    - German FEA - Substances Hazardous to Waters: Class 3 - Severe Hazard to Waters

**Hazard Statement and Occupational Control**

4,5-Dichloro-2-octyl-2H-isothiazol-3-one has harmonized classifications in the EU, as indicated in Table 2. General personal protective equipment (PPE) recommendations are presented in Table 3, below. No occupational exposure limits (OELs) were identified.

**Table 2: GHS H Statements for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5) (Pharos 2023)**

| H Statement | H Statement Details |
|---|---|
| H302 | Harmful if swallowed |
| H330 | Fatal if inhaled |
| H317 | May cause an allergic skin reaction |
| H314 | Causes severe skin burns and eye damage |
| H318 | Causes serious eye damage |
| H400 | Very toxic to aquatic life |
| H410 | Very toxic to aquatic life with long lasting effects |

**Table 3: Occupational Exposure Limits and Recommended Personal Protective Equipment for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)**

| Personal Protective Equipment (PPE) | Reference | Occupational Exposure Limits (OEL) | Reference |
|---|---|---|---|
| Face shield and safety glasses; gloves; complete body suit; respirator | PubChem 2023 | None identified | |

OEL: Occupational Exposure Limit

**Physicochemical Properties of 4,5-Dichloro-2-octyl-2H-isothiazol-3-one**

4,5-Dichloro-2-octyl-2H-isothiazol-3-one is a solid at room temperature and has low volatility. It is slightly soluble in water and the reported log $K_{ow}$ values suggest a low to moderate potential for bioaccumulation.

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

| Table 4: Physical and Chemical Properties of 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5) | | |
|---|---|---|
| **Property** | **Value** | **Reference** |
| Molecular formula | $C_{11}H_{17}Cl_2NOS$ | PubChem 2023 |
| SMILES Notation | CCCCCCCCN1C(=O)C(=C(S1)Cl)Cl | PubChem 2023 |
| Molecular weight | 282.2 | PubChem 2023 |
| Physical state | Solid | ECHA 2018a |
| Appearance | Off-white to beige, may have compact agglomerates | ECHA 2018a |
| Melting point | 410°C (EU A1/OECD Guideline 102) | ECHA 2018a |
| Boiling point | N/A – decomposes | ECHA 2018a |
| Vapor pressure | $0.98x10^{-3}$ Pa ($7.4x10^{-6}$ mmHg) at 25°C<br>$2.2x10^{-3}$ Pa ($1.7x10^{-5}$ mmHg) at 30°C<br>$4.6x10^{-3}$ Pa ($3.5x10^{-5}$ mmHg) at 30°C<br>(OECD Guideline 104)<br><br>$1.4x10^{-3}$ Pa ($1.1x10^{-5}$ mmHg) at 20°C<br>$2.7x10^{-3}$ Pa ($2.0x10^{-5}$ mmHg) at 25°C<br>(OECD Guideline 104) | ECHA 2018a |
| Water solubility | 2.3 mg/L at 10°C and pH 5<br>2.3 mg/L at 10°C and pH 7;<br>3.5 mg/L at 20°C and pH 5<br>3.1 mg/L at 20°C and pH 7;<br>5.5 mg/L at 30°C and pH 5<br>5.1 mg/L at 30°C and pH 7<br>(OECD Guideline 107)<br><br>Log $K_{ow}$ = 14 mg/L | ECHA 2018a<br><br>U.S. EPA 2020a |
| Dissociation constant | N/A (does not dissociate) | ECHA 2018a |
| Density/specific gravity | 1.32 g/cm$^3$ (OECD Guideline 109) | ECHA 2018a |
| Partition coefficient | Log $K_{ow}$ = 4.6 at 20°C and pH 5<br>Log $K_{ow}$ = 4.4 at 20°C and pH 6.3<br>Log $K_{ow}$ = 4.8 at 20°C and pH 9.1<br>(OECD Guideline 107)<br><br>Log $K_{ow}$ = 2.8 | ECHA 2018a<br><br>U.S. EPA 2020a |

**Toxicokinetics**

The toxicokinetics of 4,5-dichloro-2-octyl-2H-isothiazol-3-one have been well studied following oral and dermal exposures (ECHA 2018a,b,c). Oral absorption of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is dose-dependent and moderate (< 20%). It is extensively metabolized by ring opening de-chlorination, and subsequent metabolism to NNOMA and derivatives. The proposed metabolic pathway, as presented in the ECHA Risk Assessment Committee (RAC) background document, is shown below (ECHA 2018b). Once absorbed, it distributes to the liver, kidney and gastrointestinal tract. The main route of elimination is through the feces, which accounts for 80% of the administered test substance; most of the remainder is excreted in the urine. Dermal absorption is relatively high (39-46%), metabolism is similar to the oral route.

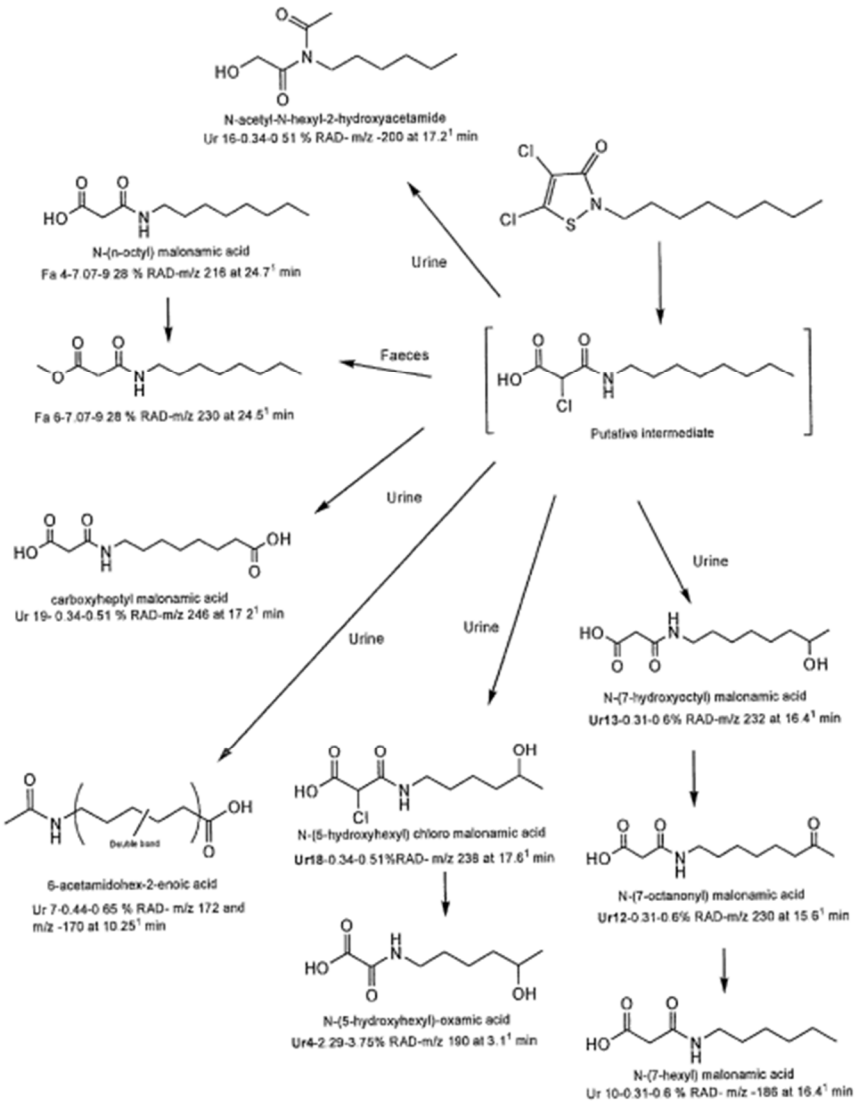



**Figure 1. Proposed Metabolic Pathway for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (ECHA 2018b)**

## Hazard Classification Summary

### Group I Human Health Effects (Group I Human)

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for carcinogenicity based on negative results in oral and dermal carcinogenicity studies with surrogates and the conclusion of the ECHA RAC that 4,5-dichloro-2-octyl-2H-isothiazol-3-one does not warrant classification for carcinogenicity. 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 high as it is based on consistently negative data for two surrogates with support from the conclusions of an authoritative body.

- 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 2018c
  - The ECHA RAC concluded that 4,5-dichloro-2-octyl-2H-isothiazol-3-one does not warrant classification for carcinogenicity based on toxicokinetic studies demonstrating that it is readily absorbed, metabolized, and eliminated, negative *in vitro* and *in vivo* genotoxicity studies, lack of evidence of an endocrine mechanism of carcinogenesis, and negative carcinogenicity studies with mixtures of related isothiazolinones (MIT and CMIT in rats and mice by the oral and dermal routes and OIT in mice by the oral route) (Klimisch score not reported).
- ECHA 2018a,b,c
  - *Oral: Surrogate: Mixture of CMIT and MIT (CAS # 55965-84-9):* In a chronic study conducted according to OECD Guideline 453, male and female Sprague-Dawley rats (10/sex/dose) were exposed to a mixture of CMIT and MIT (Kathon 866; containing 10.6% CMIT and 3.5% MIT) in drinking water at concentrations of 30, 100, or 300 mg/kg/day for 25 months (reported by ECHA to be equivalent to 0, 2.0, 6.6, and 17.2 mg/kg/day for males and 0, 3.1, 9.8, and 25.7 mg/kg/day for females. Treatment did not results in neoplastic effects, and the main treatment-related effect observed was gastric irritation (Klimisch score not reported).
  - *Dermal: Surrogate: Mixture of CMIT and MIT (CAS # 55965-84-9):* In a chronic dermal study, 40 male CD-1 mice received topical applications of 25 µL of a 0 or 400 ppm of a mixture of CMT and MIT (Kathon GC containing 1.5% a.i. as a 75%:25% mixture of CMIT:MIT) three times per week for 30 weeks. There were no neoplasms observed in the skin, liver, lung, heart, kidneys, spleen, stomach, intestines, or bones. The only effect observed was slight to moderate epidermal hyperplasia and hyperkeratosis (Klimisch score not reported).
  - *Oral: Surrogate: OIT (CAS #26530-20-1):* In a chronic study conducted prior to establishment of guidelines, male and female C57B/6xC3H/And mice received 0, 500, or 1,000 ppm OIT (purity not reported) in their feed for 78 weeks. Treatment did not result in neoplasms of the liver, lung, bladder, spleen, small intestines, kidney, thyroid, stomach, brain, prostate, ovary, mammary gland, gonads, or skin (Klimisch score not reported).

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for mutagenicity/genotoxicity based on negative results in several *in vitro* and *in vivo* mutagenicity and clastogenicity assays. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity 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 as it is based on reliable experimental 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 2018a,b,c (Note: The ECHA reviews include brief summaries of some additional studies that they state are not "key" studies. These are not included below due to the availability of a large number of reliable studies for this endpoint.)
  - *In vitro:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity and vehicle not reported) was negative in a bacterial mutagenicity assay conducted according to OECD Guideline 471 with *Salmonella typhimurium* test strains TA 1535, TA, 1537, TA 98, and TA 100 at concentrations of 0.3 to 300 µg/plate, with and without metabolic activation. Concentrations of 100 µg/plate with metabolic activation and 3-10 µg/plate without metabolic activation

produced cytotoxicity. No additional details were provided (Klimisch score not reported).

- *In vitro:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity and vehicle not reported) was negative in a bacterial mutagenicity assay conducted according to OECD Guideline 471 with *S. typhimurium* test strains TA 1535, TA 1537, TA 98, TA 100 and *Escherichia coli* $WP_2$ *uvr*A at concentrations of 1.5 to 1,500 µg/plate with and without metabolic activation. No additional details were provided (Klimisch score not reported).
- *In vitro:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity and vehicle not reported) was negative in a bacterial mutagenicity assay conducted according to OECD Guideline 471 with *S. typhimurium* test strains TA 1535, TA 1537, TA 98, TA 100 and TA102 at concentrations of 0.06 to 1.0 µg/plate without metabolic activation and 0.5 to 10 µg/plate with metabolic activation. Concentrations of 5 µg/plate without metabolic activation and 50 µg/plate with metabolic activation produced cytotoxicity. Although two unspecified strains produced increases in revertants of 1.44 fold and 1.05 fold, these small increases did not meet the criteria for a positive response (at least 2 fold increase over solvent control). No additional details were provided (Klimisch score not reported).
- *In vitro:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity and vehicle not reported) was negative in an *in vitro* chromosome aberration test conducted according to OECD Guideline 473 with Chinese hamster ovary (CHO) cells tested at concentration of 0.1 to 0.7 µg/mL without metabolic activation and 3.0 to 8.0 µg/mL with metabolic activation. Concentrations were based on results of a pretest reporting cytotoxicity at 0.25 to 0.6 µg/mL without metabolic activation and 5 to 10 µg/mL with metabolic activation. No additional details were provided (Klimisch score not reported).
- *In vitro:* 5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported, acetone solvent) was negative in an *in vitro* mammalian chromosome aberration test conducted according to OECD Guideline 473 with human primary lymphocytes tested at concentrations of 0.09 to 1.5 µg/mL with and without metabolic activation. Concentrations of 1.5 µg/mL and higher were cytotoxic. The authors stated that the positive controls were valid (Klimisch score not reported).
- *In vitro:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity and vehicle not reported) was negative in an *in vitro* mammalian cell gene mutation assay conducted according to OECD Guideline 476 with CHO cells tested at 0.005 to 0.75 µg/mL without metabolic activation and 0.5 to 25 µg/mL with metabolic activation. Cytotoxicity occurred at concentrations of 0.75 µg/mL without metabolic activation and 15 µg/mL with metabolic activation. No additional details were provided (Klimisch score not reported).
- *In vitro:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported, DMSO solvent) was negative in an *in vitro* mammalian cell gene mutation assay conducted according to OECD Guideline 476 with Chinese hamster V79 cells tested at 0.03 to 2 µg/mL without metabolic activation and 2.5 to 20 µg/mL with metabolic activation. Cytotoxicity occurred at 0.16 µg/mL without metabolic activation and 20 µg/mL with metabolic activation. The authors reported that the positive controls were valid.
- *In vivo:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity and vehicle not reported) was negative in an *in vivo* micronucleus assay conducted according to OECD Guideline 474 with male and female mice (5-9/group, strain not specified) that received a single oral dose of 60, 300, or 600 mg/kg via gavage, followed by sacrifice after 24 or 48 hours. The doses were selected based on a previous study in which 600 mg/kg/day induced toxicity and mortality in both sexes. No additional details were provided (Klimisch score not reported).
- *In vivo:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported, peanut oil vehicle) was negative in an *in vivo* chromosome aberration assay conducted according to OECD Guideline 475 with Swiss mice (5/sex/dose) that received two consecutive daily oral doses

of 0, 100, 200, or 400 mg/kg via gavage. A pretest reported mortality at doses between 750 and 1,500 mg/kg. The high dose caused a slight reduction in body weight, and clinical signs were mild (piloerection, polyurea). There were no treatment-related increases in chromosome aberrations, and the authors concluded that the substance was negative in the assay.

- *In vivo:* 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported, peanut oil vehicle) was negative in an *in vivo* unscheduled DNA synthesis (UDS) assay with Wistar rats (4/sex) that received single oral doses of 0, 1,000, or 2,000 mg/kg via gavage, followed by sacrifice at 2 or 16 hours post-treatment. Clinical signs (reduced activity, abdominal position, ruffled fur, apathy) were noted in all treated animals. There were no increases in UDS, and the authors reported the positive control was valid.

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for reproductive toxicity based on a lack of adverse effects on reproductive parameters in two dietary two-generation studies in rats. 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 as it is based on reliable experimental 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 2018a,b,c
  - In a GLP-compliant 2-generation reproductive toxicity study conducted according to a draft version of OECD Guideline 416/U.S. EPA OPPTS 870.3880, male and female Crl:CD BR rats (26/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (100% purity, vehicle not reported) in the diet at concentrations of 0, 200, 400, 800, or 3,200 ppm (equivalent to 0, 16-21, 30-41, 62-93, and 235-259 mg/kg/day according to ECHA) beginning 10 weeks prior to mating and continuing until sacrifice of parental, F1, and F2 generations. The high dose was discontinued after one generation due to offspring mortality, and the 400 ppm dose group was added (with its own control group). Treatment did not cause any mortality, clinical signs of toxicity, or effects on body weight on parental animals up to 800 ppm. At the high dose, cumulative body weight gain decreased 13-45% in both sexes during the premating period, 16-31% in females during gestation, and 8-18% in females during lactation. The incidence of paleness increased in high dose females and F1 offspring. The high dose offspring had distended abdomens and mortality was high; therefore, the offspring at this dose could not be mated to produce a second generation. There were no treatment-related clinical signs observed in F2 animals up to 400 ppm during premating in both sexes), and no effects on body weight up to 800 ppm in females. At 800 ppm, F1 males had reduced body weight gain and increased incidence of paleness. Treatment did not result in pathological changes in the reproductive organs in any group, and there were no effects on mating, fertility, gestation, lactation, or viability indices, offspring viability, estrus cycle, or sperm parameters up to 800 ppm. ECHA reported a NOAEL of 400 ppm (30-41 mg/kg/day) and LOAEL of 800 ppm (62-93 mg/kg/day) for parental toxicity based on clinical signs and body weight changes. ECHA concluded that this study does not show any evidence of a reproductive hazard through two generations at up to 800 ppm in the diet; therefore, the reproductive NOAEL is 800 ppm (62-93 mg/kg/day) (Klimisch score not reported).
  - In a 2-generation study conducted according to OECD Guideline 416, male and female Crl(WI)BR rats (24/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.1%

purity) in the diet at 100, 350, or 1,050 ppm (equivalent to 3-4, 14-16, and 57-71 mg/kg/day according to ECHA). Select F1 offspring were treated for 10 weeks after weaning and then mated to produce the F2 generation, and maternal animals and F2 offspring were sacrificed after weaning. In the F0 generation, there were statistically significant decreases in body weight and body weight gain in females periodically throughout the study, and terminal body weights were decreased at the mid and high doses (93.2% and 91.8% of controls, respectively. Also at the high dose, relative brain weights were increased in females. Pathological changes at the high dose included incidence and severity of hyperplasia of the squamous epithelium of the forestomach and lymph granulocytic inflammation of the forestomach of both sexes. Sperm count, motility, and morphology, estrus cycle, and reproductive parameters were unaffected. ECHA reported a NOAEL and LOAEL of 350 ppm and 1,050 ppm (14-16 mg/kg/day and 57-71 mg/kg/day), respectively, for systemic toxicity based on effects on body weight and body weight gain in F0 dams and effects on organ weights and stomach abnormalities in F1 dams. They reported a NOAEL of 1,050 ppm (57-71 mg//kg/day) for reproductive toxicity based on a lack of adverse effects up to the highest dose tested (Klimisch score not reported

**Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): L**
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for developmental toxicity based on results of two oral 2-generation studies in rats and several oral prenatal developmental toxicity studies in rats and rabbits that did not identify consistent effects on developmental parameters, and the conclusion of ECHA's RAC that the available data on 4,5-dichloro-2-octyl-2H-isothiazol-3-one do not support classification as a developmental toxicant. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data and the conclusions of an authoritative body.

- 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 2018a,b,c
  - In the previously described GLP-compliant 2-generation reproductive toxicity study conducted according to a draft version of OECD Guideline 416/U.S. EPA OPPTS 870.3880, male and female Crl:CD BR rats (26/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (100% purity, vehicle not reported) in the diet at concentrations of 0, 200, 400, 800, or 3,200 ppm (equivalent to 0, 16-21, 30-41, 62-93, and 235-259 mg/kg/day according to ECHA) beginning 10 weeks prior to mating and continuing until sacrifice of parental, F1, and F2 generations. The high dose was discontinued after one generation due to offspring mortality, and the 400 ppm dose group was added (with its own control group). Treatment did not cause any mortality, clinical signs of toxicity, or effects on body weight on parental animals up to 800 ppm. At the high dose, cumulative body weight gain decreased 13-45% in both sexes during the premating period, 16-31% in females during gestation, and 8-18% in females during lactation. The incidence of paleness increased in high dose females and F1 offspring. The high dose offspring had distended abdomens and mortality was high; therefore, the offspring at this dose could not be mated to produce a second generation. Histopathological changes included hyperplasia and hyperkeratosis of non-glandular mucosa of the stomach and hypertrophy and vacuolization of the adrenal cortex at the high dose. There were no treatment-related clinical signs observed in F2 animals up to 400 ppm during premating in either sex, and no effects on body weight up to 800 ppm in females. At 800

ppm, F1 males had reduced body weight gain and increased incidence of paleness. At the high dose, offspring viability was decreased. There was a dose-dependent statistically significant delay in vaginal opening in the F1 generation that was not reported for the F2 generation; because the effect occurred without effects on body weight, the authors considered it to be treatment related. Also in F1 animals, there was a statistically significant delay in preputial separation, which the authors did not consider to be treatment related because the mean number of days was comparable to the initial control group. There were no effects on anogenital distance in F2 pups. Pup body weights decreased in F1 and F2 pups at 800 ppm beginning on postnatal day 14, which ECHA notes is the approximate time when pups wean and begin to consume treated food. Gross pathological findings in F1 and F2 pups at 800 ppm included thin and watery blood, enlarged heart, pale lungs, liver, kidney and/or intestines. Relative thymus weights were decreased in both sexes at 800 ppm and 3,200 ppm for the F1 generation and 400 ppm and 800 ppm for the F2 generation; these effects corresponded to decreased cellularity in the thymus at 800 ppm and above in the F1 and F2 animals. The absolute and relative spleen weights were decreased in F1 males and females at 400 ppm and above. ECHA reported a NOAEL of 400 ppm (30-41 mg/kg/day) and LOAEL of 800 ppm (62-93 mg/kg/day) for parental toxicity based on clinical signs and body weight changes. They identified a NOAEL of 200 ppm (16-21 mg/kg/day) and LOAEL of 400 ppm (30-41 mg/kg/day) for offspring systemic toxicity based on decreased thymus weights in the F2 generation, but noted that effects may be secondary to reduced weight gain at the time of weaning (Klimisch score not reported).

- In the previously described 2-generation study conducted according to OECD Guideline 416, male and female Crl(WI)BR rats (24/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.1% purity) in the diet at 100, 350, or 1,050 ppm (equivalent to 3-4, 14-16, and 57-71 mg/kg/day according to ECHA). Select F1 offspring were treated for 10 weeks after weaning and then mated to produce the F2 generation, and maternal animals and F2 offspring were sacrificed after weaning. In the F0 generation, there were statistically significant decreases in body weight and body weight gain in females periodically throughout the study, and terminal body weights were decreased at the mid and high doses (93.2% and 91.8% of controls, respectively. Also at the high dose, relative brain weights were increased in females. Pathological changes at the high dose included incidence and severity of hyperplasia of the squamous epithelium of the forestomach and lymph granulocytic inflammation of the forestomach of both sexes. In the F1 offspring, body weights were decreased in both sexes at the high dose and the authors also reported mal-rotated legs, absent/reduced tail, and reduced anus size in four pups. Absolute spleen and thymus weights were decreased and relative brain weights were increased in high dose F1 offspring, which the authors concluded is related to reduced body weights. There was a slight delay in preputial separation in high dose males, which the authors also considered related to reduced body weights. There were no effects on vaginal opening or on anogenital distance (measured only in F2 pups). In terms of systemic evaluations of the F1 generation, effects in the high dose group included decreased absolute prostate weights in males, decreased terminal body weight at necropsy, decreased absolute brain and ovary weights, increased relative kidney weights and adrenal weights in females, and increased incidence and severity of hyperplasia of the squamous epithelium of the forestomach and lymph granulocytic inflammation of the forestomach in both sexes. In the F2 generation, there were decreases in body weight in both sexes at the high dose, and absolute spleen weight in both sexes and relative spleen weight in females were decreased at the mid and high doses. The authors considered these changes secondary to reduced body weights. Affected tail apex and reduced size/opaqueness of left eye were also noted in four pups. ECHA reported

a NOAEL and LOAEL of 350 ppm and 1,050 ppm (14-16 mg/kg/day and 57-71 mg/kg/day), respectively, for systemic toxicity based on effects on body weight and body weight gain in F0 dams and effects on organ weights and stomach abnormalities in F1 dams. They reported a developmental toxicity NOAEL of 350 ppm (14-16 mg/kg/day) and LOAEL of 1,050 ppm (57-71 mg/kg/day) based on reduced body weights in both sexes at the high dose, noting that the effects on the spleen at 350 ppm were only noted in F2 pups and were of uncertain biological significance (Klimisch score not reported).

- In a GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant CrlLCD BR rats (25/dose) received 0, 10, 30 ,100, or 300 mg/kg/day 4,5-dichloro-2-octyl-2H-isothiazol-3-one (98.8% purity, corn oil vehicle) via gavage on gestation days 6-15 and were sacrificed on gestation day 20. The high dose group was terminated prior to completion of the study due to severe maternal toxicity (weight loss, soft feces, diarrhea, altered posture, mortality). There were no effects on gross pathology, numbers of early or late resorptions, live fetuses per litter, fetal body weight, or sex ratio. Treatment caused an increase in the number of litters with fetuses with wavy ribs at 100 mg/kg/day, and there was a dose-related increase in total skeletal variations that was significant at the high dose. There was a slight, non-significant increase in total malformations in the two highest dose groups. ECHA reported a NOAEL and LOAEL of 10 mg/kg/day and 30 mg/kg/day, respectively, for maternal toxicity and a NOAEL and LOAEL of 30 mg/kg/day and 100 mg/kg/day, respectively, for developmental toxicity, noting that the study suggests a developmental delay that was not accompanied by increases in structural abnormalities (Klimisch score not reported).
- In a developmental toxicity study in rats that was reported with limited details, the oral NOAELs were 10 mg/kg/day for maternal toxicity (based on scant/soft feces, diarrhea, and reduced feed consumption and weight gain) and 30 mg/kg/day for developmental toxicity (based on increased number of litters with fetuses with wavy ribs at 100 mg/kg/day) toxicity (Klimisch score not reported).
- In a prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant Crl: CD(SD)BR rats (25/dose) received a preformulation of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (C9211M, 48.9% a.i. in xylene, methylcellulose vehicle) via gavage at doses corresponding to 0, 11.2, 33.7, or 112.4 mg/kg/day of the active ingredient on gestation days 6-15 and were sacrificed on gestation day 20. Maternal effects included clinical signs and reduced body weight, at the two highest doses and mortality at the high dose. The authors attributed bent ribs and limbs observed at the high dose to maternal toxicity. Developmental effects included increases in the percentage of fetuses with skeletal malformations at the two highest doses and reduced fetal body weights at the high dose. There were no effects on external or soft tissue malformations. ECHA reported a NOAEL of 11.2 mg/kg/day and a LOAEL of 33.7 mg/kg/day for both maternal toxicity and developmental toxicity (Klimisch score not reported).
- In a prenatal developmental toxicity conducted according to U.S. EPA OPP 83-3, pregnant female New Zealand White rabbits (20/dose) received a preformulation of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (C9211, 40% a.i. in xylene, methylcellulose vehicle) via gavage at doses corresponding to 0, 5, 25, or 70 mg/kg/day of the active ingredient via gavage on gestation days 7-19 and were sacrificed on gestation day 29. Maternal toxicity including clinical signs and a dose-related decrease in body weight gain (statistically significant at the two highest doses) occurred at all doses. There was a significant decrease in the number of live fetuses per litter and a non-significant increase in abortions at the high dose. There were no effects on the number of fetal skeletal or soft tissue malformations, but the number of fetuses available for evaluation was too low for evaluation. ECHA reported a NOAEL and

LOAEL of 5 mg/kg/day and 25 mg/kg/day, respectively, for maternal toxicity and a NOAEL and LOAEL of 25 mg/kg/day and 70 mg/kg/day, respectively, for developmental toxicity (Klimisch score not reported).

- In a prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant New Zealand White rabbits (24/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.1% purity, peanut oil vehicle) in their feed at 0, 125, 500, or 2,000 ppm (corresponding to 0, 2, 10, and 44 mg/kg/day according to ECHA) on gestation days 6-28, when they were sacrificed. Maternal effects included decreased food consumption and body weight gain at the high dose. Developmental effects included retinal abnormalities (detached retina and/or choroid) at the high dose, which the authors noted did not occur in any other developmental toxicity studies. There were no effects on external or visceral malformations, and a non-statistically significant increase in the incidence of fetuses with extra ribs at the mid and high doses, which fell outside the historical control range. ECHA reported a NOAEL and LOAEL of 10 mg/kg/day and 44 mg/kg/day, respectively, for both maternal and developmental toxicity (Klimisch score not reported).
- In a developmental toxicity study in rabbits that was reported with limited details, the oral NOAELs was 25 mg/kg/day based on a lack of malformations (including eye malformations). However, the number of fetuses available for evaluation was insufficient (Klimisch score not reported).
- Another oral prenatal developmental toxicity study (OECD Guideline 414) study with New Zealand White rabbits reported no maternal or developmental toxicity at oral doses up to 20 mg/kg/day; however, this study is not included in the weight of evidence due to low reliability (Klimisch 3, not reliable).

- ECHA 2018c
  - ECHA's RAC reviewed the proposed classification for 4,5-dichloro-2-octyl-2H-isothiazol-3-one (ECHA 2018a), and agreed that the available data do not support classification for developmental toxicity. They noted that treatment produced maternal toxicity at doses of 30 mg/kg/day and above in rats and 5 mg/kg/day and above in rabbits, but even severe maternal toxicity did not have impacts on the ability to carry the pregnancy to term the numbers or early or late resorptions, liver fetuses per litter, fetal body weight, sex ratio, or external or soft tissue malformations. Only one study in rabbits reported effects on body weight and live fetuses at a dose that caused significant maternal mortality, and although rat studies reported statistically significant skeletal malformations and variations, these occurred mostly at doses with severe maternal toxicity including mortality, and ECHA did not consider effects on the incidence of bent ribs at a non-maternally toxic dose to be sufficient for classification purposes. ECHA also noted that effects on incidences of variations in rabbits were also insufficient for classification based on inconsistency of the effects, lack of dose response, and relation to the historical control data. Therefore, they concluded that the compound does not warrant classification for developmental toxicity.

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Moderate for endocrine activity based on effects on preputial separation and vaginal opening in oral 2-generation studies in rats and positive results in a number of *in vitro* assays. GreenScreen® criteria classify chemicals as a Moderate hazard for endocrine activity when there is evidence of endocrine activity (CPA 2018b). The preliminary score of Moderate is not modified to a High, because there are no related adverse health effects. The confidence in the score is low as effects in the 2-generation studies were inconsistent and sometimes attributed to body weight effects, and because the relevance of the *in vitro* assays is uncertain.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* TEDX - Potential Endocrine Disruptors: Potential Endocrine Disruptor
- U.S. EPA 2023a
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one was active in 10/21 estrogen receptor (ER) assays, 9/15 androgen receptor (AR) assays, 2/2 steroidogenesis assays, and 9/18 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century.
- ECHA 2018a,b,c
  - In a previously-described GLP-compliant 2-generation reproductive toxicity study conducted according to a draft version of OECD Guideline 416/U.S. EPA OPPTS 870.3880, male and female Crl:CD BR rats (26/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (100% purity, vehicle not reported) in the diet at concentrations of 0, 200, 400, 800, or 3,200 ppm (equivalent to 0, 16-21, 30-41, 62-93, and 235-259 mg/kg/day according to ECHA) beginning 10 weeks prior to mating and continuing until sacrifice of parental, F1, and F2 generations. There was a dose-dependent statistically significant delay in vaginal opening in the F1 generation that was not reported for the F2 generation; because the effect occurred without effects on body weight, the authors considered it to be treatment related. Also in F1 animals, there was a statistically significant delay in preputial separation, which the authors did not consider to be treatment related because the mean number of days was comparable to the initial control group. There were no effects on anogenital distance in F2 pups (Klimisch score not reported).
  - In a 2-generation study conducted according to OECD Guideline 416, male and female Crl(WI)BR rats (24/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.1% purity) in the diet at 100, 350, or 1,050 ppm (equivalent to 3-4, 14-16, and 57-71 mg/kg/day according to ECHA). Select F1 offspring were treated for 10 weeks after weaning and then mated to produce the F2 generation, and maternal animals and F2 offspring were sacrificed after weaning. There was a slight delay in preputial separation in high dose males, which the authors also considered related to reduced body weights. There were no effects on vaginal opening or on anogenital distance (measured only in F2 pups) (Klimisch score not reported).
  - In a GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant CrlLCD BR rats (25/dose) received 0, 10, 30 ,100, or 300 mg/kg/day 4,5-dichloro-2-octyl-2H-isothiazol-3-one (98.8% purity, corn oil vehicle) via gavage on gestation days 6-15 and were sacrificed on gestation day 20. The high dose group was terminated prior to completion of the study due to severe maternal toxicity (weight loss, soft feces, diarrhea, altered posture, mortality). There were no effects on sex ratio (Klimisch score not reported).

**<u>Group II and II* Human Health Effects (Group II and II* Human)</u>**
***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): vH**
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very High for acute toxicity based on a harmonized classification of H330 - Fatal if inhaled in the EU with support from an inhalation $LC_{50}$ (primarily vapor) of 0.26 mg/L/4h in rats, which is below the guidance value of 0.5 mg/L/4h vapor for

GHS Category 1 classification, and 0.164 mg/L/4h (aerosol) in rats, which is between the guidance values of 0.05 and 0.5 mg/L/4h mist for GHS Category 2. GreenScreen® criteria classify chemicals as a Very High hazard for acute toxicity when they are classified to GHS Category 1 or 2, carry the hazard statement H330, and inhalation $LC_{50}$ values are less than 0.5 mg/L/4h (vapor) (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data and an authoritative list.

- Authoritative and Screening Lists
    - *Authoritative:* EU - GHS (H-Statements) Annex 6 Table 3-1: H330 - Fatal if inhaled [Acute toxicity (inhalation) - Category 1 or 2].
    - *Authoritative:* EU - GHS (H-Statements) Annex 6 Table 3-1: H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4].
    - *Screening:* GHS - New Zealand: Acute inhalation toxicity category 2.
    - *Screening:* GHS – Australia: H330 - Fatal if inhaled [Acute toxicity (inhalation) - Category 1 or 2].
    - *Screening:* GHS – Korea H330 - Fatal if inhaled [Acute toxicity (inhalation) - Category 2].
    - *Screening:* GHS – Korea: H311 - Toxic in contact with skin [Acute toxicity (dermal) - Category 3].
    - *Screening:* GHS – Australia: H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4].
    - *Screening:* GHS – Korea: H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4].
    - *Screening:* GHS - New Zealand: Acute oral toxicity category 4.
- ECHA 2018a,b,c
    - *Oral*: $LD_{50}$ (male and female Crl:CD BR rats) = 1,636 mg/kg (OECD Guideline 401) (Klimisch 1, reliable without restriction)
        - *4,5-Dichloro-2-octyl-2H-isothiazol-3-one is classified to GHS Category 4 based on an oral $LD_{50}$ > 300 mg/kg and $\leq$ 2,000 mg/kg (UN 2021).*
    - *Oral*: $LD_{50}$ (male and female Crl:CD-1(ICR) BR mice) = 567 mg/kg (OECD Guideline 401) (Klimisch 1, reliable without restriction)
        - *4,5-Dichloro-2-octyl-2H-isothiazol-3-one is classified to GHS Category 4 based on an oral $LD_{50}$ > 300 mg/kg and $\leq$ 2,000 mg/kg (UN 2021).*
    - *Oral*: $LD_{50}$ (male and female Wistar rats) > 500 mg/kg and < 2,000 mg/kg (OECD Guideline 423) (Klimisch 1, reliable without restriction)
        - *4,5-Dichloro-2-octyl-2H-isothiazol-3-one is classified to GHS Category 4 based on an oral $LD_{50}$ > 300 mg/kg and $\leq$ 2,000 mg/kg (UN 2021).*
    - *Dermal*: $LD_{50}$ (New Zealand White rabbits) > 2,000 mg/kg for a pre-formulation of 32.6% in xylene (equivalent to > 652 mg/kg a.i.) (OECD Guideline 402) (Klimisch 1, reliable without restriction)
        - *This study is insufficient for classification purposes because the highest dose (a.i.) is less than the dermal guidance value of 2,000 mg/kg for classification (UN 2021)*
    - *Dermal*: $LD_{50}$ (male and female Wistar rats) > 2,000 mg/kg (OECD Guideline 402) (Klimisch 1, reliable without restriction).
        - *4,5-Dichloro-2-octyl-2H-isothiazol-3-one is not classified under GHS (Category 4 or higher) based on an oral $LD_{50}$ > 2,000 mg/kg (UN 2021).*
    - *Inhalation*: $LC_{50}$ (male and female Crl:CD BR rats) = 0.26 mg/L/4h (mixture of aerosol and vapor) (OECD Guideline 403) (Klimisch 1, reliable without restriction)
        - *This value is compared to a guidance value of < 0.5 mg/L/4h (vapor) for Category 1 (UN 2021), as ECHA states that the test substance was significantly volatilized. Therefore, 4,5-dichloro-2-octyl-2H-isothiazol-3-one is classified to Category 1 based on this study.*

- *Inhalation*: $LC_{50}$ (male and female Wistar rats) = 0.164 mg/L/4h (aerosol) (OECD Guideline 403) (Klimisch 1, reliable without restriction)
  - *4,5-Dichloro-2-octyl-2H-isothiazol-3-one is classified to GHS Category 2 based on an $LC_{50}$ > 0.05 mg/L/4h and < 0.5 mg/L/4h (vapor) (UN 2021).*

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very High for systemic toxicity (single dose) based on evidence of irreversible respiratory corrosion noted at concentrations less than the guidance values of 10 mg/L (vapor) and 1 mg/L (aerosol) in several acute inhalation studies with 4,5-dichloro-2-octyl-2H-isothiazol-3-one, warranting GHS Category 1 classification. GreenScreen® criteria classify chemicals as a Very High hazard for systemic toxicity (single dose) when they are classified to GHS Category 1 (CPA 2018b). The confidence in the score is low as the study summaries did not clearly differentiate between effects seen in surviving animals and those that died and did not specifically discuss reversibility of effects, and because some of the studies involved a mixture with xylene.

- 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 2018a,b,c
  - *Oral*: In a test conducted according to OECD Guideline 401, male and female Crl:CD BR rats (6/sex/dose) received 500, 750, 1,000, 1,500, or 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) in corn oil via gavage and were observed for 14 days. Deaths increased with dose (details not provided). Animals displayed irritation around the anal-genital area, passiveness, scant feces, and/or stained muzzle. Body weights decreased in surviving males at doses of 750 mg/kg and higher. Gross pathological effects included viscous material in the cecum, intestines, and stomach, black material or foci adhered to stomach mucosa, reddened stomach and intestinal mucosa, and mottled liver. The study summary does not specify whether these effects occurred in surviving animals or those that died. The stomach walls were thickened in surviving animals (Klimisch 1, reliable without restriction).
  - *Oral*: In a test conducted according to OECD Guideline 401, male and female Crl:CD-1(ICR) BR mice (6/sex/dose) received 100, 250, 500, 1,000, or 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) in corn oil via gavage and were observed for 14 days. Deaths increased with dose (details not provided). There were no effects on body weight. Clinical signs included soft and/or scant feces, passiveness, tremors, and ataxia in males at 1,000 mg/kg and higher and females at 500 mg/kg and higher. Gross pathological effects included reddened glandular portion of the stomach and intestines, black material in the stomach, and mottled liver (Klimisch 1, reliable without restriction).
  - *Oral*: In a test conducted according to OECD Guideline 423, male and female Wistar rats (3/sex/dose) received 200, 500, or 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) in peanut oil via gavage (observation period unspecified). All animals at the high dose died. Dose-depended clinical signs included lethargy, abdominal breathing, and nasal discharge at 200 mg/kg, lethargy, abdominal breathing, toe walking, and piloerection at 500 mg/kg, and lethargy, abdominal breathing, gasping, nasal discharge, piloerection, toe walking, salivation, diarrhea, and unusual locomotion at 2,000 mg/kg. The authors reported unspecified gross pathological changes in lung, liver, kidneys, and spleen of animals at the high dose (Klimisch 1, reliable without restriction).

- *Dermal*: In a test conducted according to OECD Guideline 402, male and female New Zealand White rabbits (6/sex/dose) received dermal applications of 2,000 mg/kg of a formulation containing 32.6% 4,5-dichloro-2-octyl-2H-isothiazol-3-one in xylene (equivalent to 652 mg/kg a.i.) for 24 hours under occlusion. There were no deaths. Animals displayed ataxia, reduced body weights, decreased feed consumption, scant feces, and passiveness. Gross pathological effects included red fluid filled thoracic cavity and clear fluid filled abdominal cavity, as well as erythema, edema, pocketing edema, eschar, and blanching of the skin (Klimisch 1, reliable without restriction).
- *Dermal*: In a test conducted according to OECD Guideline 402, male and female Wistar rats (5/sex/dose) received a single application of 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) under unspecified exposure conditions. There were no deaths, and the only treatment-related effects reported were rough coat and erythema corresponding to skin lesions including cutaneous thickening, alopecia, and erythema in treated animals (Klimisch 1, reliable without restriction).
- *Inhalation*: In a test conducted according to OECD Guideline 403, male and female Crl:CD BR rats (6/sex/concentration) were exposed to 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) as a mixture of aerosol and vapor (exact ratio unknown, but significantly vaporized according to ECHA) at 0.23, 0.12, 0.46, or 0.20 mg/L for 4 hours (nose only), and were observed for 14 days. Deaths occurred at all concentrations, without a dose response. Clinical signs at all concentrations included gasping and slight to severe rales, indicative of respiratory irritation, unkempt appearance, red stained eyes and muzzle, scant feces, and yellow stained anogenital area. Gross pathological evaluations found scattered incidences of red pinpoint foci on the lungs and gas filled stomachs. ECHA considered the results of this study to illustrate respiratory irritation due to the corrosive properties of the test substance, and attributed the deaths to corrosive properties (Klimisch 1, reliable without restriction).
- *Inhalation*: In a test conducted according to OECD Guideline 403, male and female Wistar rats (5/sex/concentration) were exposed to 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported, in DMSO solvent) aerosol at concentrations of 0.142, 0.221, and 0.289 mg/L aerosol for 4 hours and were observed for 14 days. Mortality was 40%, 70%, and 50% at the low, mid, and high concentrations, respectively. Clinical signs included lethargy, tremors, abdominal breathing, gasping, and nasal irritation. Gross pathological changes in animals that died from all groups included vascular/inflammatory changes in the lungs. Effects were inconsistent and not dose-dependent. ECHA noted that the exposure concentration could have been higher because the authors used only gravimetric methods which do not measure adsorbed vapors, and that the presence of DMSO as a solvent could have impacted absorption of the test substance (Klimisch 1, reliable without restriction).
- *Inhalation*: In a study designed to evaluate respiratory irritation, male Swiss Webster mice (number not specified) were exposed head-only to 32, 75, 86, 112, 167, 184, or 198 µg/L of a formulation containing 30% 4,5-dichloro-2-octyl-2H-isothiazol-3-one in xylene, followed by a 15 minute post-exposure period. At 86 µg/L and higher, the respiratory rates were modestly decreased, with a 33% decrease in respiratory rate at concentrations of 112 µg/L and higher. The xylene control group did not show similar effects (Klimisch score not reported).
- *Inhalation*: In a 3-month inhalation study conducted according to OECD Guideline 413 that is described in detail below for repeated dose toxicity, male and female CRL:CD BR rats 32/sex/dose) were exposed to an aerosol of a preformulation of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (C-9211 M HQ, 32.6% in o-xylene) (corresponding to 0, 0.02, 0.63, and 6.72 mg/m$^3$ a.i. based on analytical measurements) via nose only inhalation for 6 hours/day,

5 days/week. Clinical signs included rales, gasping, and dyspnea. At the high concentration, lung weights significantly increased due to edema in the lungs, and histopathology revealed treatment-related effects indicative of irritation to the nose, larynx, and lungs at the mid and high concentrations. Effects resolved following a 6-month follow up in recovery animals (Klimisch score not reported).

- ECHA 2018c
    - In its evaluation of 4,5-dichloro-2-octyl-2H-isothiazol-3-one, the ECHA RAC concluded that based on studies demonstrating respiratory clinical signs in rats exposed by inhalation and histopathological changes presumed to be irreversible, 4,5-dichloro-2-octyl-2H-isothiazol-3-one warrants classification with the supplemental hazard statement EUH071 (corrosive to the respiratory tract). They note that Category 3 (H335) classification for respiratory tract irritation is not appropriate, as the observed effects were too severe.
- Based on the weight of evidence, ToxServices assigned a score of Very high for this endpoint based on evidence of irreversible respiratory corrosion noted at concentrations less than the guidance values of 10 mg/L (vapor) and 1 mg/L (aerosol) in several acute inhalation studies with 4,5-dichloro-2-octyl-2H-isothiazol-3-one, warranting GHS Category 1 classification. ToxServices did not assign GHS Category 3 classification, because Category 3 classification applies to transient (i.e., reversible) effects. As noted by the ECHA RAC, the histopathological effects seen in acute inhalation studies with rats were too severe to be considered transient effects; therefore, the RAC assigned a classification of EUH071, which is a supplemental classification applied in the EU for substances that are acutely toxic due to respiratory corrosion. ToxServices agrees with ECHA's conclusion that the effects were too severe to support Category 3 classification; however, the EUH071 hazard statement does not correspond to a GreenScreen® classification. Therefore, ToxServices assigned a Very High because the irreversible effects occurred at concentrations below the guidance values for Category 1.

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for systemic toxicity (repeated dose) based on the lack of systemic effects supporting classification fore repeated dose toxicity from several subchronic oral, dermal, and inhalation studies and the ECHA RAC's conclusion that the available data on 4,5-dichloro-2-octyl-2H-isothiazol-3-one do not support classification as target organ toxicant following repeated exposures. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when adequate data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data and the conclusions of an authoritative body.

- 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 2018a,b,c
    - *Oral*: In a 4-week study conducted according to Japanese guidelines, male and female SD rats (10/sex/dose) received 0, 20, 100, or 500 mg/kg/day 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.5% purity, olive oil vehicle) via gavage for 28-days, followed by a 28-day recovery period. There were no treatment-related effects at 20 mg/kg/day. Three females at the high dose died. Treatment caused decreased body weight gain and feed consumption and alterations in absolute and relative organ weights and urinalysis at the high dose, mostly in male animals. At 100 mg/kg/day and higher, males had a significant but slight decrease in relative liver weight. Slight but significant changes in clinical chemistry and hematology

were also measured at 100 mg/kg/day and higher. Gross pathological changes included thickening of the mucosa of the non-glandular stomach and small and large intestine at the high dose, and atrophy of the liver in males at the high dose. Histopathological changes included slight changes in the stomach and small intestine and increased fat content in the adrenals at 100 mg/kg/day and higher. ECHA reported a NOAEL of 20 mg/kg/day and a LOAEL of 100 mg/kg/day (Klimisch score not reported).

- *Oral*: In a 3-month study conducted according to OECD Guideline 408, male and female Crl:CD BR rats (10/sex/dose) received 4,5-dichloro-2-octyl-2H-isothiazol-3-one (98.8% purity) in their feed at 0, 100, 500, 1,000, or 4,000 ppm, which ECHA states is equivalent to 6.2, 32.5, 60.7, and 248.2 mg/kg/day for males and 7.2, 36.7, 74.7, and 278.4 mg/kg/day for females. Effects noted at 1,000 ppm and higher in the feed included significant reductions in body weight gain, feed consumption, and triglyceride levels in females, and minimal histopathological changes in the forestomach in one animal of each sex at 1,000 ppm. Treatment with 4,000 ppm in the diet caused forestomach irritation with effects ranging from minimal hyperkeratosis and slight epithelial hyperplasia to erosion/ulcerations with associated inflammation and edema of the submucosa. There were no effects on urinalysis, and hematological effects at the high dose were considered to be related to blood loss caused by the gastric lesions. Serum triglyceride levels were reduced in females at 1,000 ppm both sexes at 4,000 ppm; there were several additional changes in clinical chemistry, but the authors noted many were of minimal magnitude. ECHA identified a NOAEL of 500 ppm (32.5-36.7 mg/kg/day) and a LOAEL of 1,000 ppm (60.7-74.7 mg/kg/day) for this study (Klimisch score not reported)
- *Oral*: In a 3-month study conducted according to OECD Guideline 408, male and female Wistar rats (10/sex/dose) were administered 0, 35, 70, or 105 mg/kg/day 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.4% purity, peanut oil vehicle) via gavage for 90 days, followed by a 28-day recovery period for separate groups of control and high dose animals. Final body weights were significantly decreased in mid and high dose males (by approximately 10% and 15%, respectively, compared to the control group) and high dose females (by approximately 15% compared to the control group); these effects were associated with reduced feed consumption. Other changes in the mid and high dose animals included significantly increased relative testes and adrenal weights and significantly reduced testicular sperm head counts; there were no morphological changes, and effects resolved after the recovery period. The authors reported a NOAEL of 35 mg/kg/day and a LOAEL of 70 mg/kg/day, but stated that these effects may be the result of stress due to local irritation and reduced food consumption (Klimisch score not reported).
- *Oral*: In a 3-month study conducted according to OECD Guideline 407 with male and female beagle dogs, animals (4/sex/dose) received diets containing 4,5-dichloro-2-octyl-2H-isothiazol-3-one (98.42% purity) at 100, 300, or 1,500 ppm (equivalent to 3.4, 10.2, and 47.5 mg/kg/day for males and 3.4, 10.1, and 45.9 mg/kg/day for females according to ECHA). There were no treatment-related effects at the low and mid doses. At the high dose, body weight and food consumption decreased significantly in females, and all dogs except for one male lost weight during the treatment period. There were some changes in hematological and clinical chemistry that the authors considered to be related to body weight decreases, and an increased incidence and severity of thymic atrophy in females. ECHA identified a NOAEL of 300 ppm (10.2-10.1 mg/kg/day) and a LOAEL of 1,500 ppm (47.5-45.9 mg/kg/day) for this study (Klimisch score not reported).
- *Oral*: In a 3-month study conducted according to OECD Guideline 409 with beagle dogs, animals (4/sex/dose) received a diet containing 100, 300, 1,500, 3,000, or 4,500 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.1% purity) (equivalent to 0, 2, 5, 27, 61, and 88

mg/kg/day for males and 0, 2, 6, 35, 61, and 74 mg/kg/day for females according to ECHA). Treatment with the two highest doses resulted in food scatter, vomiting, and reduced body weights the authors considered to be due to palatability issues and irritation. The high dose treatment also caused clinical chemistry changes (reduced cholesterol, phospholipids, total protein, albumin, globulin, and calcium levels, and increased AST, ALT and GLT-activities) and statistically significant reductions in absolute thymus, thyroid, epididymides, heart, liver, and prostate weights. Some unspecified clinical chemistry changes also occurred at 1,500 ppm, which the authors considered to be secondary to the poor nutritional state of the animals. Although increased transaminase activities in males the 3,000 and 4,500 mg/kg/day groups were not associated with pathological changes, the authors considered the effect to be treatment-related. ECHA reported a NOAEL of 1,500 ppm (27/35 mg/kg/day) and a LOAEL of 3,000 ppm (61 mg/kg/day) (Klimisch score not reported).

- *Dermal*: In a 21-day dermal study conducted prior to establishment of current guidelines, male and female New Zealand White rabbits (6/sex/dose) were administered 0, 1, or 5 mg/kg/day of a preformulation of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (C-9211M, 35% in mixed xylene in acetone) (corresponding to 0.35 and 1.75 mg/kg/day a.i.) to the skin under semiocclusion 5 days/week for 21 days (total of 15 doses). Treatment did not cause any systemic effects, but the low dose produced skin irritation including hyperplasia, hyperkeratosis or parakeratosis of epidermis, increased amounts of inflammatory cell infiltration in the dermis, and focal dermal hemorrhage. No additional details were provided (Klimisch score not reported).
- *Dermal*: In a 28-day dermal study that was conducted according to OECD Guideline 410, male and female Wistar rats (20/sex/dose for control and high dose groups, 10/sex/dose for low and mid dose groups) received dermal applications of 0, 3, 15, or 16/30 mg/kg day for 28 days. The high dose of 60 mg/kg/day was discontinued after 8 days, and resumed with 30 mg/kg/day after day 21. Effects included severe edema and erythema leading to mortality, significant reductions in body weight, hematological alterations (reduced red blood cell count, hemoglobin and hematocrit, increased eosinophil granulocyte count and red blood cell distribution volume), and there was a slight increase in relative spleen and adrenal weights. The authors noted that the effects were related to the immunological and stress response secondary to the skin corrosion caused by the test substance (Klimisch score not reported). ECHA reported a NOAEL of 3 mg/kg/day and a LOAEL of 15 mg/kg/day.
- *Inhalation*: In a 3-month inhalation study conducted according to OECD Guideline 413, male and female CRL:CD BR rats 32/sex/dose) were exposed to an aerosol of a preformulation of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (C-9211 M HQ, 32.6% in o-xylene) (corresponding to 0, 0.02, 0.63, and 6.72 $mg/m^3$ a.i. based on analytical measurements) via nose only inhalation for 6 hours/day, 5 days/week. Recovery groups were maintained without treatment for 6 months and 1 year. There was no evidence of systemic toxicity. Clinical signs included rales, gasping, and dyspnea. At the high concentration, lung weights significantly increased due to edema in the lungs, and histopathology revealed treatment-related effects indicative of irritation to the nose, larynx, and lungs at the mid and high concentrations. Effects resolved by the 6- month follow up in the recovery animals. In the proposed classification report, ECHA reported a NOAEC of 0.02 $mg/m^3$ and a LOAEC of 63 $mg/m^3$ based on histopathological changes in the respiratory tract. The ECHA RAC report 0.02 $mg/m^3$ as the LOAEC based on similar effects in a small number of animals in the low concentration group (Klimisch score not reported).

- ECHA 2018c

- ECHA's RAC reviewed the proposed classification for 4,5-dichloro-2-octyl-2H-isothiazol-3-one (ECHA 2018a), and agreed that the available data do not support classification for specific target organ toxicity-repeated exposure. Although studies generally reported effects below the guidance values for classification, the ECHA RAC notes that the reported effects, including hematological changes, are mostly related or secondary to gastrointestinal, dermal, and respiratory irritation, which are addressed by other classifications. Furthermore, the RAC concluded that effects measured on organ weights in several studies are not sufficient to warrant classification.

**Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L):** DG
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Data Gap for neurotoxicity (single dose) based on a lack of sufficient data for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2018a,b,c
  - *Oral*: In a test conducted according to OECD Guideline 401, male and female Crl:CD BR rats (6/sex/dose) received 500, 750, 1,000, 1,500, or 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) in corn oil via gavage and were observed for 14 days. Deaths increased with dose (details not provided). There were no clinical signs or gross pathological findings related to neurotoxicity (Klimisch 1, reliable without restriction).
  - *Oral*: In a test conducted according to OECD Guideline 401, male and female Crl:CD-1(ICR) BR mice (6/sex/dose) received 100, 250, 500, 1,000, or 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) in corn oil via gavage and were observed for 14 days. Deaths increased with dose (details not provided). Clinical signs included passiveness, tremors, and ataxia in males at 1,000 mg/kg and higher and females at 500 mg/kg and higher. There were no gross pathological effects on neuronal tissues (Klimisch 1, reliable without restriction).
  - *Oral*: In a test conducted according to OECD Guideline 423, male and female Wistar rats (3/sex/dose) received 200, 500, or 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) in peanut oil via gavage (observation period unspecified). All animals at the high dose died. Dose-depended clinical signs included lethargy, and abdominal breathing at 200 mg/kg, lethargy, abdominal breathing, toe walking, and piloerection at 500 mg/kg, and lethargy, abdominal breathing, gasping, nasal discharge, piloerection, toe walking, salivation, diarrhea, and unusual locomotion at 2,000 mg/kg. There were no gross pathological effects on neuronal tissues (Klimisch 1, reliable without restriction).
  - *Dermal*: In a test conducted according to OECD Guideline 402, male and female New Zealand White rabbits (6/sex/dose) received dermal applications of 2,000 mg/kg of a formulation containing 32.6% 4,5-dichloro-2-octyl-2H-isothiazol-3-one in xylene (equivalent to 652 mg/kg a.i.) for 24 hours under occlusion. There were no deaths. Animals displayed ataxia, and passiveness. There were no gross pathological effects on neuronal tissues (Klimisch 1, reliable without restriction).
  - *Dermal*: In a test conducted according to OECD Guideline 402, male and female Wistar rats (5/sex/dose) received a single application of 2,000 mg/kg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) under unspecified exposure conditions. There were no deaths, and there were no clinical signs or gross pathological findings related to neurotoxicity (Klimisch 1, reliable without restriction).
  - *Inhalation*: In a test conducted according to OECD Guideline 403, male and female Crl:CD

BR rats (6/sex/concentration) were exposed to 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) as a mixture of aerosol and vapor (exact ratio unknown, but significantly vaporized according to ECHA) at 0.23, 0.12, 0.46, or 0.20 mg/L for 4 hours (nose only), and were observed for 14 days. Deaths occurred at all concentrations, without a dose response. There were no clinical signs or gross pathological findings related to neurotoxicity (Klimisch 1, reliable without restriction).

  - *Inhalation*: In a test conducted according to OECD Guideline 403, male and female Wistar rats (5/sex/concentration) were exposed to 4,5-dichloro-2-octyl-2H-isothiazol-3-one (purity not reported, in DMSO solvent) aerosol at concentrations of 0.142, 0.221, and 0.289 mg/L aerosol for 4 hours and were observed for 14 days. Mortality was 40%, 70%, and 50% at the low, mid, and high concentrations, respectively. Clinical signs included lethargy, and tremors. There were no gross pathological findings related to neurotoxicity. ECHA noted that the exposure concentration could have been higher because the authors used only gravimetric methods which do not measure adsorbed vapors, and that the presence of DMSO as a solvent could have impacted absorption of the test substance (Klimisch 1, reliable without restriction).

- Based on the weight of evidence, a Data Gap was assigned. Some acute studies reported potential signs of neurotoxicity, such as lethargy, tremors, but such reports are too inconsistent to consistently conclude as evidence of neurotoxicity. In addition, the data are insufficient to conclusively support a Low.

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for neurotoxicity (repeated dose) based on a lack of neurotoxicity reported in a subchronic oral study in rats at oral doses up to 105 mg/kg/day, which exceeds the oral guidance value of 100 mg/kg/day for GHS classification, with support from a lack of neurobehavioral effects at up to 88 mg/kg/day in a 90-day study in dogs and 30 mg/kg/day in a 28-day dermal study in rats. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when adequate data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental 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 2018a,b,c
  - *Oral*: In a previously-described 3-month study conducted according to OECD Guideline 408, male and female Wistar rats (10/sex/dose) were administered 0, 35, 70, or 105 mg/kg/day 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.4% purity, peanut oil vehicle) via gavage for 90 days, followed by a 28-day recovery period for separate groups of control and high dose animals. Evaluations included a functional observation battery for neurobehavioral assessment, and there were no treatment-related effects. Therefore, ToxServices identified a neurotoxicity NOAEL of 105 mg/kg/day for this study (Klimisch score not reported).
  - *Dermal*: In a previously-described 28-day dermal study that was conducted according to OECD Guideline 410, male and female Wistar rats (20/sex/dose for control and high dose groups, 10/sex/dose for low and mid dose groups) received dermal applications of 0, 3, 15, or 16/30 mg/kg day for 28 days. Parameters evaluated included neurobehavioral abnormalities, and the study summary did not report any effects. No additional details were provided (Klimisch score not reported).
- ECHA 2018a,b,c, U.S EPA 2015b
  - *Oral*: In the U.S. EPA's evaluation of the 3-month study conducted according to OECD

Guideline 409 in which beagle dogs (4/sex/dose) received a diet containing 100, 300, 1,500, 3,000, or 4,500 4,5-dichloro-2-octyl-2H-isothiazol-3-one (97.1% purity) (equivalent to 0, 2, 5, 27, 61, and 88 mg/kg/day for males and 0, 2, 6, 35, 61, and 74 mg/kg/day for females according to ECHA), U.S. EPA stated that there were no neurobehavioral findings. Therefore, ToxServices identified a neurotoxicity NOAEL of 88/74 mg/kg/day

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of High for skin sensitization based on results of a local lymph node assay and guinea pig maximization test, as well as human data indicating a high frequency of reactions, supporting GHS Category 1A classification. GreenScreen® criteria classify chemicals as a High hazard for skin sensitization when they are classified to GHS Category 1A (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data and human evidence.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) Annex 6 Table 3-1: H317 - May cause an allergic skin reaction [Skin sensitization - Category 1].
  - *Screening:* GHS – Korea: H317 - May cause an allergic skin reaction [Skin sensitization - Category 1].
  - *Screening:* GHS - New Zealand: Skin sensitisation category 1.
  - *Screening:* GHS – Australia: H317 - May cause an allergic skin reaction [Skin sensitization - Category 1].
- ECHA 2018a,b,c
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) was positive in a local lymph node assay conducted according to OECD Guideline 429 (GLP status not specified) with mice (n=4/group, sex and strain not specified) tested at concentrations of 0.005%, 0.01%, 0.1%, 0.25%, and 0.5% in acetone:olive oil, 4:1. The stimulation indices (SI) were 0.8, 1.1, 11.6, 25.7 and 27.0, respectively, and the authors calculated an EC3 of 0.03% for this study (Klimisch score not reported).
    - *According to GHS criteria (UN 2021), an EC3 value $\leq$ 2% in an LLNA corresponds to GHS Category 1A.*
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) was positive in a guinea pig maximization test conducted according to OECD Guideline 406 (GLP status not specified). The animals (10/sex/dose, 5/sex/control) were tested at an intradermal induction concentration of 5% in propylene glycol with and without 1:1 Freund's Complete Adjuvant (FCA), and topical induction concentrations of 25% in 80% alcohol. Following challenge with 5% test substance in acetone, 60% and 45% of the animals displayed positive responses after 24 and 48 hours, respectively (Klimisch score not reported).
    - *According to GHS criteria, positive responses in > 30% of animals at > 1% intradermal induction dose in a guinea pig maximization test corresponds to GHS Category 1B.*
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) was positive in a second guinea pig maximization test conducted according to OECD Guideline 406 (GLP status not specified). The animals (10/sex/dose, strain not specified) were tested at intradermal induction concentrations of 0.01%, 0.02%, or 0.03% in mineral oil, with or without FCA, followed by topical induction and challenge with the same concentrations. The lowest concentration produced positive responses in 75% and 55% of the animals at 24 and 48 hours, respectively. The middle concentration produced positive responses in 95% of the animals at both observations, and the high concentration produced positive responses in all

of the animals at both observations (Klimisch score not reported).
    - *According to GHS criteria, positive responses in > 30% of animals at ≤ 0.1% intradermal induction dose in a guinea pig maximization test corresponds to GHS Category 1A.*
- Several human case and clinical studies report sensitization effects, with an induction threshold at or below 0.025% (higher if diluted in corn oil or petrolatum). A brief summary of these studies as described by ECHA is summarized below.
    - Beginning three weeks after introduction of a new biocide (30% 4,5-dichloro-2-octyl-2H-isothiazol-3-onein xylene) to a finishing agent in a textile factory, 8/9 workers developed itchy red eruptions on exposed skin. Open patch testing with six volunteers was strongly positive for the biocide and finishing agent.
    - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one was sensitizing at concentrations ≥ 0.025% in a repeat insult patch test (RIPT)
    - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one was sensitizing at 0.025% in ethanol in a 24-hour occlusive patch test with individuals that previously tested positive in an RIPT.
    - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one was sensitizing at 0.035% in ethanol in 24- and 48-hour occlusive patch tests with individuals that previously tested positive in an RIPT

**Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L):** DG
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Data Gap for respiratory sensitization based on a lack of sufficient data for this endpoint.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2023
    - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one does not contain any structural alerts for respiratory sensitization (Appendix D)
- No data were identified for the target compound for this endpoint. Therefore, ToxServices attempted to evaluate the respiratory sensitization potential of 4,5-dichloro-2-octyl-2H-isothiazol-3-one 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). 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). 4,5-Dichloro-2-octyl-2H-isothiazol-3-one does not contain any structural alerts, but is a skin sensitizer based on positive experimental 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): vH**

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very High for skin irritation/corrosivity based on a harmonized classification of H314 - Causes severe skin burns and eye damage in the EU, with support from several rabbit studies reporting tissue destruction following a 4-hour exposure, indicating that GHS Category 1 classification is warranted. GreenScreen® criteria classify chemicals as a Very High hazard for skin irritation/corrosivity when they are classified to GHS Category 1 with the hazard statement H314 (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data and an authoritative listing.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) Annex 6 Table 3-1: H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1A or 1B or 1C].
  - *Screening:* GHS – Australia H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1A or 1B or 1C].
  - *Screening:* GHS – Korea: H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1].
  - *Screening:* GHS - New Zealand Skin corrosion category 1C.
- ECHA 2018a,b,c
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (as a 32.6% preformulation in xylene) was corrosive to the skin of New Zealand White rabbits (n=6) in a test conducted according to OECD Guideline 404. Application of 0.5 mL for 4 hours (skin preparation and coverage unspecified) produced severe edema and erythema, with mean 24/48/72 hour scores (average of all animals) of 4.0 for erythema and 3.9 for edema. Effects were not reversible within 14 days, with 5/6 animals showing scar formation (Klimisch score not reported).
  - In a test conducted according to OECD Guideline 404, application of a formulation containing 20% 4,5-dichloro-2-octyl-2H-isothiazol-3-one in phenoxypropanol (propylene glycol phenyl ether) to the skin of one rabbit for 4 hours skin (preparation and coverage unspecified) produced irreversible skin irritation (concave eschar) within 48 hours, which persisted through day 14, when a veterinarian confirmed corrosive findings (ulceration and erosion) (Klimisch score not reported).
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) was corrosive in an OECD Guideline 404 dermal irritation study with New Zealand White rabbits (n=3). Application of an unspecified volume for 4 hours produced mean (of all animals) 24/48/72 hour scores of 2.3 for erythema and 2.2 for edema. The severity of the skin lesions increased over time, and the effects did not resolve within 14 days. Therefore, the authors predicted full-thickness destruction of the skin and classified the substance as corrosive.
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (purity not reported) was predicted to be non-corrosive in an *in vitro* human epidermal construct test conducted according to OECD Guideline 431 using the EPIDERM™ test system. Application of the undiluted test substance reduced the viability by less than 10%. ECHA notes that there was no post-exposure incubation time and the maximum exposure time was 60 minutes. Therefore, they did not consider the results of this test to be sufficient to disregard *in vivo* data (Klimisch score not reported).
  - The ECHA evaluation also included supplemental data supporting the skin irritation endpoint (Klimisch scores not reported). These data are briefly summarized below:
    - Guinea pig maximization tests reported very faint to faint irritation after exposure to 0.02%, with the highest non-irritating concentration of 0.01% in mineral oil. In range-finding studies, concentrations of 0.1% in aqueous ethanol and 0.03% in acetone were irritating.

- Human clinical studies with 4,5-dichloro-2-octyl-2H-isothiazol-3-one in various vehicles reported no irritation up to 1,000 ppm in petrolatum and corn oil, and a threshold at or near 0.025% to 0.035% in ethanol.

**Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): vH**
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very High for eye irritation/corrosivity based on a harmonized classification of H318 - Causes serious eye damage. GreenScreen® criteria classify chemicals as a Very High hazard for eye irritation/corrosivity when they are classified with H318 (CPA 2018b). The confidence in the score is high as it is based on an authoritative listing.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) Annex 6 Table 3-1 H318 - Causes serious eye damage [Serious eye damage/eye irritation - Category 1].
  - *Screening:* GHS - New Zealand Serious eye damage category 1
- ECHA 2018a,b,c
  - No ocular irritation data are available, but because it is corrosive to the skin, it is expected to be corrosive to the eyes.

**Ecotoxicity (Ecotox)**

**Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): vH**
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very High for acute aquatic toxicity based on its harmonized classification of H400 - Very toxic to aquatic life in the EU and the lowest L/$EC_{50}$ values of 2.7 µg/L (0.0027 mg/L) in fish, 2.1 µg/L (0.0021 mg/L) in invertebrates, and 0.48 µg/L (0.00048 mg/L) in algae. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when they are classified with H400 and the lowest L/$EC_{50}$ values are 1 mg/L or lower (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data and an authoritative listing.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) Annex 6 Table 3-1: H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1].
  - *Screening:* GHS – Korea: H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1].
  - *Screening:* GHS - New Zealand Hazardous to the aquatic environment - acute category 1
- ECHA 2018a,b,c (Note: Klimisch scores were not provided for these studies.)
  - 96-hour $LC_{50}$ (*Oncorhynchus mykiss*, rainbow trout) = 2.7 µg/L (EPA FIFRA-72.1)
  - 96-hour $LC_{50}$ (*Lepomis macrochirus*, bluegill sunfish) = 14 µg/L (EPA FIFRA-72.1)
  - 96-hour $LC_{50}$ (*Cyprinodon variegatus*, sheepshead minnow) = 20.5 µg/L (EPA FIFRA-72.3)
  - 96-hour $LC_{50}$ (*Takifugu rubripes*, Japanese blowfish) = 5.66 µg/L (OECD Guideline 203)
  - 96-hour $LC_{50}$ (*O. mykiss*, rainbow trout) = 7.8 µg/L (OECD Guideline 203)
  - 96-hour $LC_{50}$ (*C. variegatus*, sheepshead minnow) = 7.3 µg/L (OECD Guideline 203)
  - 48-hour $EC_{50}$ (*Daphnia magna*, water flea) = 5.2 µg/L (EPA FIFRA-72.2)
  - 48-hour $EC_{50}$ (*D. magna*, water flea) = 9.7 µg/L (OECD Guideline 202)
  - 96-hour $EC_{50}$ (*Mysidopsis bahia*, mysid shrimp) = 4.7 µg/L (EPA FIFRA-72.3)
  - 48-hour $EC_{50}$ (*Crassostrea virginica*, American oyster) = 2.1-3.2 µg/L (EPA FIFRA-72.3)
  - 48-hour $EC_{50}$ (*Mytilus edulis*, bay mussel) = 411 µg/L (EPA OPPTS 850.1055)
  - 72-hour $ErC_{50}$ (*(Scenedesmus subspicatus*, green algae) = 25 µg/L (OECD Guideline 201/EPA OPPTS 850.5400)

- 24/96-hour $ErC_{50}$ (*Navicula pelliculosa*, diatom) = 1.6 µg/L (EPA OPPTS 850.5400/OECD Guideline 201)
- 24/120-hour $ErC_{50}$ (*Skeletonema costatum*, diatom) = 0.48 µg/L (EPA FIFRA-123.2)
- 72-hour $ErC_{50}$ (*Phaeodactylum tricornutum*, marine diatom) = 25 µg/L (OECD Guideline 201/EPA OPPTS 850.5400
- 72-hour $EC_{50}$ (*Lemna gibba*, duckweed) = 206 µg/L (OECD Guideline 221/EPA OPPTS 850.4400, TSCA 797.1160, FIFRA 122.2/122.3, EC 67/548/EEC)

**Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): vH**
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very High for chronic aquatic toxicity based on chronic NOECs as low as 0.43 µg/L (0.00043 mg/L) in fish, 0.4 µg/L (0.0004 mg/L) in daphnia, and 0.34 µg/L (0.0003 mg/L) in algae. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic NOECs are 0.1 mg/L and lower (CPA 2018b). The confidence in the score is high as it is based on reliable experimental 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 2018a,b,c (Note: Klimisch scores were not provided for these studies.)
  - 97-day NOEC (*O. mykiss*, rainbow trout) = 0.56 µg/L (OECD Guideline 210)
  - 35-day NOEC (*C. variegatus*, sheepshead minnow) = 6.0 µg/L (OECD Guideline 203)
  - 35-day NOEC (*Brachydanio rerio*, zebra fish) = 0.43 µg/L (OECD Guideline 210)
  - 21-day NOEC (*D. magna*, water flea) = 0.63 µg/L (EPA FIFRA 72-4)
  - 21-day NOEC (*D. magna*, water flea) = 0.4 µg/L (OECD Guideline 211)
  - 28-day NOEC (*Americamysis bahia*, mysid) = 0.63 µg/L (EPA OPPTS 850.1350)
  - 72-hour NOErC (*(S. subspicatus*, green algae) = <15 µg/L (OECD Guideline 201/EPA OPPTS 850.5400)
  - 24/96-hour NOErC (*N. pelliculosa*, diatom) = 0.34 µg/L (EPA OPPTS 850.5400/OECD Guideline 201)
  - 24/120-hour NOErC (*S. costatum*, diatom) = 0.48 µg/L (EPA FIFRA-123.2)
  - 72-hour NOErC (*P. tricornutum*, marine diatom) = 4.3 µg/L (OECD Guideline 201/EPA OPPTS 850.5400
  - 72-hour NOEC (*L. gibba*, duckweed) = 4.54 µg/L (OECD Guideline 221/EPA OPPTS 850.4400, TSCA 797.1160, FIFRA 122.2/122.3, EC 67/548/EEC)

## Environmental Fate (Fate)

**Persistence (P) Score (vH, H, M, L, or vL):** *L*
4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for persistence based on experimental half-lives up to 2 days in soil, which is predicted to be the dominant compartment for 4,5-dichloro-2-octyl-2H-isothiazol-3-one. GreenScreen® criteria classify chemicals as a Low hazard for persistence when the half-life in soil is less than 15 days (CPA 2018b). The confidence in the score is low as the available studies did not report half-lives for ultimate degradation.

- 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 2018a,b,c
  - In a hydrolysis test performed according to OECD Guideline 111 and U.S. EPA guidelines, 4,5-dichloro-2-octyl-2H-isothiazol-3-one had a half-life of 71 days at a pH of 7 at 25°C.

The calculated half-life at 12°C was 178-201 days. Degradation products present above 10% included 2-chloro-2-(noctylcarbamoyl)-1-ethene sulfonic acid, 1-chloro2-(n-octylcarbamoyl)-1-ethene sulfonic acid, and N-(n-octyl) propiolic acid amide.

- In a second hydrolysis test performed according to OECD Guideline 111, 4,5-dichloro-2-octyl-2H-isothiazol-3-one had a half-life of 25 days at a pH of 7 at 25°C. The calculated half-lives at pH 7 and 12°C were 41-47 days.
- In a photolysis study conducted according to U.S. EPA guidelines, the half-life in sunlight was 13 days (38 days at 12°C) and the half-life in the dark was 80 days (225 days at 12°C). The only degradation product over 10% was N-(N-octyl) oxamic acid (NNOOA).
- In a test conducted according to draft OECD/U.S. EPA guidelines, the half-life for 4,5-dichloro-2-octyl-2H-isothiazol-3-one at 25°C in summer sunlight was 7.6 days in buffer and 7.9 days in pond water. The substance was rapidly mineralized; only 50% of the radioactivity remained after 19 days. The only degradation product present over 10% was NNOOA.
- In a study conducted according to draft OECD Guidelines, the photolysis half-life for 4,5-dichloro-2-octyl-2H-isothiazol-3-one was 4.5-56 days with 24 hours sunlight at 50°N (Artificial sunlight) depending on the season (9-112 days at 12 hour sunlight).
- 4,5-Dichloro-2-octyl-2H-isothiazol-3-one was not readily biodegradable in a test conducted according to OECD Guideline 301B. The test substance underwent virtually no biodegradation; however, it was inhibitory to the inoculum at a concentration of 32 ppm.
- In a ready biodegradability test conducted according to OECD Guideline 301B, 4,5-dichloro-2-octyl-2H-isothiazol-3-one was borderline inhibitory to the inoculum at 25 mg/L, and therefore, no reliable results could be obtained.
- The degradation product of 4,5-dichloro-2-octyl-2H-isothiazol-3-one, NNOMA, was readily biodegradable and met the 10-day window in a test conducted according to OECD Guideline 301B.
- In a 144-hour aerobic stimulation study conducted according to a draft version of OECD Guideline 309 using estuarine surface water without sediment, the half-life of in estuarine surface water without sediment in water was 20-32 hours at 10°C and 4.2-12 hours at 18°C. The calculated half-lives at 9°C and 12°C were 8.7-35 hours and 6.8-28 hours, respectively. The main degradation product was NNOOA and other degradation products present were 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid, 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid and 1,2-dichloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid.
- In a simulation study in a freshwater-sediment system (draft OECD Guideline 308), 4,5-dichloro-2-octyl-2H-isothiazol-3-one had a half-life in the water phase of 1.6 days at 12°C under aerobic conditions and 0.17 days at 12°C under anaerobic conditions. The compound was not detected in the sediment, but the authors stated the rate is valid for the whole system. After 101 days, 62% (aerobic) and 50% (anaerobic) of the radioactivity was in the bound residues fraction. Reported degradation products included N-(n-octyl) malonamic acid (NNOMA), N-(n-octyl) acetamide, 3,3'dithiobis-(n-octyl)-3-chloropropenamide, and 2-chloro-3-(formyldithio)-N-octylpropenamide.
- In a simulation study in seawater and sediment that was conducted according to U.S. EPA guidelines, the half-lives under aerobic and anaerobic conditions were less than one hour at 25°C and the calculated half-lives at 9°C were less than 3.6 hours. Most of the radioactivity was measured in the sediment. Up to 8.2% of the applied radioactivity was detected in the water phase, but not in the form of the target compound. After 30 days under aerobic conditions, 64% of the radioactivity was detected in the bound residues fraction and 10-20% (aerobic) and 7-8% (anaerobic) was detected as carbon dioxide. Degradation products were not characterized but the authors stated they were likely NNOMA and N-(n-octyl)

acetamide, which were detected in an additional study that focused on identifying degradation products. Based on a separate study demonstrating that the target compound may be quantitatively extracted from marine stable and does not degrade in a sterile environment, the authors concluded that the residue in sediment in non-sterile systems corresponded to degradation products.
    - The half-lives of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in soil under aerobic conditions were 2.0 and 0.58-1.1 days at 6°C and 25°C, respectively, in tests conducted according to U.S. EPA guidelines. The substance rapidly incorporated into bound residues, and 41-54% of the radioactivity was measured in post-extraction solids. The major degradation product was carbon dioxide (11-21% of the radioactivity), and although one other product was present at greater than 10%, the study did not include definitive degradation identification.
    - In a natural water/sediment simulation study conducted according to a draft version of OECD Guideline 308, the primary degradation half-life of 4,5-dichloro-2-octyl-2H-isothiazol-3-one was 1.2-1.5 days (2.5 days at 12°C). In river and pond systems, 27% and 30%, respectively, of the radioactivity was detected as carbon dioxide and the target dissipated rapidly from water, with less than 50% remaining in water after two days. Up to 57-64% of the residues were bound in sediment at 61 days, and only 2-3% were extractable, indicating that the residues were substances other than the target. There were no degradation products composing more than 9% of the radioactivity, and the authors concluded that the substance is ultimately mineralized or incorporated into natural substances such as humic acids and humins.
    - An adsorption/desorption test conducted according to U.S. EPA guidelines found that 4,5-dichloro-2-octyl-2H-isothiazol-3-one adsorbs to sludge and is unlikely to remain in water.
    - Adsorption constants of 5,659-25,236 L/kg in soil and 17,232-28,320 L/kg (outlier of 38237 L/kg) in sediment indicate strong sorption to soil and sediment and low desorption potential.
    - Volatilization from water is considered negligible based on the vapor pressure of 0.0014 Pa at 20°C and Henry's Law Constant of 0.21 Pa $m^3$/mol.
- ECHA 2018c
    - The ECHA RAC concluded that 4,5-dichloro-2-octyl-2H-isothiazol-3-one cannot be classified as readily biodegradable because it is inhibitory to the inoculum. Although it has very short primary half-lives in water, soil, and sediment, mineralization is limited, and one of the degradation products fulfills criteria for classification as hazardous in the environment. Therefore, the substance cannot be classified as rapidly degradable.
- NICNAS 2019
    - "The chemicals in this group are biodegradable at expected environmental exposure concentrations".
- U.S. EPA 2020a
    - U.S. EPA reports the following half-lives in various media:
        - Hydrolysis half lives of 260 days (pH 4), 71 days (pH 7), and 3.5 days (pH 9) with degradation products of sulfinic acid and propiolic acid amine
        - Photodegradation half-life of 17.2 days in water with degradation products of NNOOA and NNOMA
        - Anaerobic degradation half-life of < 1 hour in seawater-sediment with degradation products of NNOMA and NNOOA
        - Anaerobic half-life of 0.33 days in freshwater-sediment
        - Anaerobic half-life of 0.04 days in seawater-sediment with degradation products of NNOMA and NNOAA
        - Anaerobic half-life of 2.9 days in non-sterile freshwater with degradation products of

2-(n-octyl) carbamoyl-2-chloroethylene sulfonic acid and NNOOA
    - Anaerobic half-life of 0.86 days in freshwater-sediment
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one degrades to several products that are straight chains that biodegrade to acetic acid, formic acid, and carbon dioxide.
- U.S. EPA 2017
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that 4,5-dichloro-2-octyl-2H-isothiazol-3-one is not expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 70% will partition to soil with a half-life of 75 days, 17.4% will partition to water with a half-life of 37.5 days, and 2.24% will partition to soil with a half-life of 337.5 days (Appendix E).
- Based on the weight of evidence, ToxServices assigned a score of Low. Ready biodegradation tests for 4,5-dichloro-2-octyl-2H-isothiazol-3-one cannot be used for classification, because the substance is toxic to the inoculum. However, its primary degradation product is readily biodegradable . Modeling predicts that 4,5-dichloro-2-octyl-2H-isothiazol-3-one will partition mainly to soil. The experimental half-lives for 4,5-dichloro-2-octyl-2H-isothiazol-3-one in soil were 2.0 and 0.58-1.1 days at 6°C and 25°C, respectively, which falls into the range for a Low (< 15 days in soil). The primary degradation half-lives in sediment, water, and air also fall below the cut-offs of 16 days, 16 days and 2 days, respectively. These experimental half-lives take precedence over modeled half-lives by EPI Suite™, neither of which represents half-lives of ultimate degradation. Therefore, ToxServices assigned a Low, but confidence is reduced because the available studies did not report half-lives for ultimate degradation.

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Very Low for bioaccumulation based on a BCF of 13 for 4,5-dichloro-2-octyl-2H-isothiazol-3-one, estimated from a measured total BCF for the target and metabolites and analytical measurements of the target compound. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the BCF is less than 100 (CPA 2018b). The confidence in the score is low as it is based on an estimate from a total residue BCF, and the available studies did not specifically measure BCFs for the target compound.

- 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 2018a,b,c
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one has a log $K_{ow}$ of 4.4 at pH 6.3 and 20°C (OECD Guideline 107)
  - In a bioaccumulation study conducted according to U.S. EPA guidelines with *Lepomis machrochirus* (bluegill sunfish), the total steady-state BCF based on 14C residues (target and degradants/metabolites) was 56-660 for whole fish and the steady state BCF based on total 14C residues was 750. Because 4,5-dichloro-2-octyl-2H-isothiazol-3-one is less than 1% of the radioactivity by day 28, the authors multiplied the highest 14C BCF of 1,300 by 1% to estimate a BCF of less than 13 for 4,5-dichloro-2-octyl-2H-isothiazol-3-one.
  - In a bioaccumulation study conducted according to Guidelines of Japanese Ministry of International Trade and Industry, the 14C-BCF values were 198-1,126 and calculated steady state BCF were 713-735. The authors noted that the 14C residue comprises several compounds and therefore, the BCF values incorporate degradation products in fish. This study did not include degradation product identification.
  - In a bioaccumulation study in oysters (*Crassostrea virginica*) following U.S. EPA guidelines and OECD Guideline 305E, the BCF was 44, based on total 14C residues. ECHA notes that

14C-labelled degradation products might have been incorporated into the tissues.

- U.S. EPA 2020b
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one has a log $K_{ow}$ of 2.8.
  - Measured BCFs in fish were 660 (edible), 200 (non-edible), and 660 (whole fish). No additional details were provided.
    - ToxServices notes that the brief summary provided does not specify if these values represent the target compound or the target and metabolites.
- U.S. EPA 2017
  - BCFBAF predicts a BCF/BAF of 1.196 using the regression based model based on a measured log $K_{ow}$ of 4.4, and a BCF of 590.4 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix E).
- ECHA 2018c
  - The ECHA RAC noted that the BCF values that include degradants and metabolites are greater than 500, but that 4,5-dichloro-2-octyl-2H-isothiazol-3-one itself is likely to have a lower bioaccumulation potential due to metabolism. They concluded that "a conclusion on the bioaccumulation potential of DCOIT and its metabolites cannot be reached".
- NICNAS 2019
  - Octylisothiazolinone preservatives are not expected to bioaccumulate. Although the log $K_{ow}$ for 4,5-dichloro-2-octyl-2H-isothiazol-3-one is greater than the cutoff for categorization, it has a high reactivity with biomolecules and data indicate that it undergoes biotransformation in fish.
- Based on the weight of evidence, ToxServices assigned a score of Very Low. Two log $K_{ow}$ values have been reported for 4,5-dichloro-2-octyl-2H-isothiazol-3-one and correspond to conflicting scores; the log $K_{ow}$ of 2.8 reported by U.S. EPA (2020) corresponds to a Very Low, but the log $K_{ow}$ of 4.4 reported by ECHA (2018a,b,c) corresponds to a Moderate. Several bioaccumulation studies are available, and the highest values based on radioactivity are > 500 to 1,000, which corresponds to a Moderate. However, most of these values are based on total radioactivity, and as noted by NICNAS (2019) and the ECHA RAC (2018c), 4,5-dichloro-2-octyl-2H-isothiazol-3-one undergoes metabolism in fish and therefore total radioactivity includes both the target and metabolites. Studies reported by U.S. EPA (2019) did not specify if the values correspond to total radioactivity or radioactivity of the target compound; therefore, ToxServices did not weigh these values heavily in the assessment. The ECHA RAC concluded that data are insufficient to make a conclusion on the bioaccumulation potential, but NICNAS concluded that bioaccumulation is not expected due to the metabolism in fish. Based on a study demonstrating that only 1% of the radioactivity remaining in fish was attributable to 4,5-dichloro-2-octyl-2H-isothiazol-3-one, the estimated BCF for the target itself was less than 13. Based on the estimated BCF of 13 for the target and the conclusion made by NICNAS that the compound is not bioaccumulative due to metabolism, ToxServices assigned a Very Low.

## Physical Hazards (Physical)

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for reactivity based on its structure that does not indicate the potential for explosive or oxidizing properties and its history of safe use. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when available data indicate that they do not warrant GHS classification for any of the reactivity sub-endpoints (CPA 2018b). The confidence in the score is low because no experimental data are available.

- Authoritative and Screening Lists

  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2018a,b,c
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one does not emit flammable gas on contact with water based on experience in handling and use.
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one does not contain any functional groups that contribute to explosivity, and the oxygen balance number and exothermic decomposition energy are below the threshold for explosive substances.
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one is not oxidizing because the oxygen balance shows a negative oxygen count.

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

4,5-Dichloro-2-octyl-2H-isothiazol-3-one was assigned a score of Low for flammability based on negative results in a test for flammability of solids and a history of use indicating it is not pyrophoric. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data are available and indicate that they are not GHS classified as a flammable or pyrophoric solid (CPA 2018b). The confidence in the score is high because it is based in part on experimental flammability 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 2018a,b,c
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one was not flammable in a test conducted according to EU Method A10.
  - 4,5-Dichloro-2-octyl-2H-isothiazol-3-one is not pyrophoric based on experience in handling and use.

## Use of New Approach Methodologies (NAMs)[10] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* data for endocrine activity and genotoxicity, and *in silico* modeling for respiratory sensitization, bioaccumulation, and persistence. NAMs are non-animal alternatives 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 2020b, 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 5, Type I (input data) uncertainties in 4,5-dichloro-2-octyl-2H-isothiazol-3-one's NAMs dataset include a lack of sufficient experimental data for endocrine activity and respiratory sensitization, and a lack of validated test methods for respiratory sensitization. 4,5-Dichloro-2-octyl-2H-isothiazol-3-one's Type II (extrapolation output) uncertainties include the inability of *in vitro* genotoxicity assays to mimic *in vivo* conditions, uncertainty regarding the *in vivo* relevance of *in vitro* endocrine activity assays, and that OECD Toolbox only identifies structural alerts and ECHA's guidance on respiratory sensitization does not evaluate non-immunologic mechanisms. Some of 4,5-dichloro-2-octyl-2H-isothiazol-3-one's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

| **Table 5: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses** | |
|---|---|
| **Uncertainty Analyses (OECD 2020)** | |
| **Type I Uncertainty: Data/Model Input** | **Endocrine activity**: *In vivo* studies provide inconsistent results for endocrine activity and did not measure all critical hormone levels.<br><br>**Respiratory sensitization**: No experimental data are available and there are no validated test methods. |
| **Type II Uncertainty: Extrapolation Output** | **Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[11].<br><br>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* |

[10] 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).

[11] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427

| | | |
|---|---|---|
| | metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[12]<br><br>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[13].<br><br>**Endocrine activity:** The *in vivo* relevance of EDSP Tox 21 screening assays is unknown due to lack of consideration of metabolism and other toxicokinetic factors. EDSP Tox 21 assays do not cover all critical endocrine pathways.<br><br>**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. | |
| **Endpoint** | **NAMs Data Available and Evaluated? (Y/N)** | **Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)** |
| Carcinogenicity | No | N/A |
| Mutagenicity | **Yes** | *In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay |
| Reproductive toxicity | No | N/A |
| Developmental toxicity | No | N/A |
| Endocrine activity | **Yes** | *In vitro* high throughput data: EDSP Tox 21 screening assays/ |
| Acute mammalian toxicity | No | N/A |
| Single exposure systemic toxicity | No | N/A |
| Repeated exposure systemic toxicity | No | N/A |
| Single exposure neurotoxicity | No | N/A |
| Repeated exposure neurotoxicity | No | N/A |
| Skin sensitization | No | N/A |
| Respiratory sensitization | **Yes** | *In silico* modeling: OECD Toolbox structural alerts/Danish QSAR |
| Skin irritation | No | |
| Eye irritation | No | N/A |

[12] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[13] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

| | | |
|---|---|---|
| Acute aquatic toxicity | No | N/A |
| Chronic aquatic toxicity | No | N/A |
| Persistence | **Yes** | *In silico* modeling: EPI Suite™ Non-animal testing: OECD 301 biodegradation tests, OECD 301 hydrolysis tests, and OECD 308 and 309 and U.S. EPA guideline simulation tests. |
| Bioaccumulation | **Yes** | *In silico* modeling: EPI Suite™ |

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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 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GREEN SCREEN FOR SAFER CHEMICALS

## GreenScreen® Score Inspector

**Table 1: Hazard Table**

| | Group I Human | | | | | Group II and II* Human | | | | | | | | | Ecotox | | Fate | | Physical | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Carcinogenicity | Mutagenicity/Genotoxicity | Reproductive Toxicity | Developmental Toxicity | Endocrine Activity | Acute Toxicity | Systemic Toxicity | | Neurotoxicity | | Skin Sensitization* | Respiratory Sensitization* | Skin Irritation | Eye Irritation | Acute Aquatic Toxicity | Chronic Aquatic Toxicity | Persistence | Bioaccumulation | Reactivity | Flammability |
| **Table 2: Chemical Details** | | | | | | | S | R * | S | R * | * | * | | | | | | | | |
| **Inorganic Chemical?** / **Chemical Name** / **CAS#** | C | M | R | D | E | AT | STs | STr | Ns | Nr | SNS* | SNR* | IrS | IrE | AA | CA | P | B | Rx | F |
| No / 4,5-Dichloro-2-octyl-2H-isothiazol-3-one / 64359-81-5 | L | L | L | L | *M* | vH | *vH* | L | | L | H | DG | vH | vH | vH | vH | *L* | *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 | Yes | Yes | No |
| 3 | STOP | | | | | | |
| 4 | STOP | | | | | | |

**Table 4**

| Chemical Name | Preliminary GreenScreen® Benchmark Score |
|---|---|
| 4,5-Dichloro-2-octyl-2H-isothiazol-3-one | 2 |

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

**Table 6**

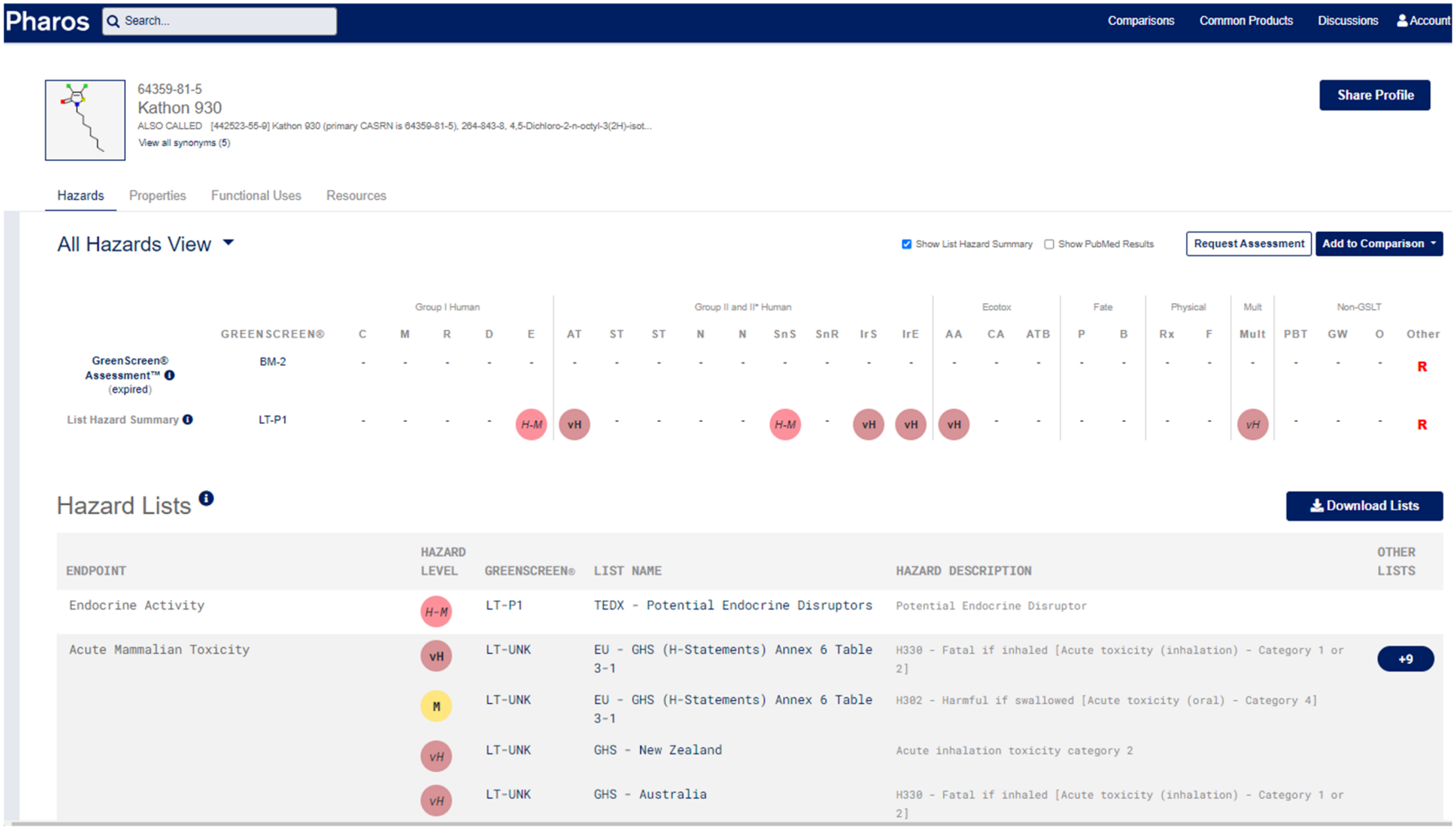
| Chemical Name | Final GreenScreen® Benchmark Score |
|---|---|
| 4,5-Dichloro-2-octyl-2H-isothiazol-3-one | 2 |

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

**Table 5: Data Gap Assessment Table**

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

## APPENDIX C: Pharos Output for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)



Pharos Search... Comparisons Common Products Discussions Account

64359-81-5
Kathon 930
ALSO CALLED [442523-55-9] Kathon 930 (primary CASRN is 64359-81-5), 264-843-8, 4,5-Dichloro-2-n-octyl-3(2H)-isot...
View all synonyms (5)

Share Profile

Hazards | Properties | Functional Uses | Resources

All Hazards View

Show List Hazard Summary | Show PubMed Results | Request Assessment | Add to Comparison

| | | Group I Human | | | | | Group II and II* Human | | | | | | | | | Ecotox | | | Fate | | Physical | | Mult | Non-GSLT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GREENSCREEN® | C | M | R | D | E | AT | ST | ST | N | N | SnS | SnR | IrS | IrE | AA | CA | ATB | P | B | Rx | F | Mult | PBT | GW | O | Other |
| GreenScreen® Assessment™ (expired) | BM-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | R |
| List Hazard Summary | LT-P1 | - | - | - | - | H-M | vH | - | - | - | - | H-M | - | vH | vH | vH | - | - | - | - | - | - | vH | - | - | - | R |

## Hazard Lists

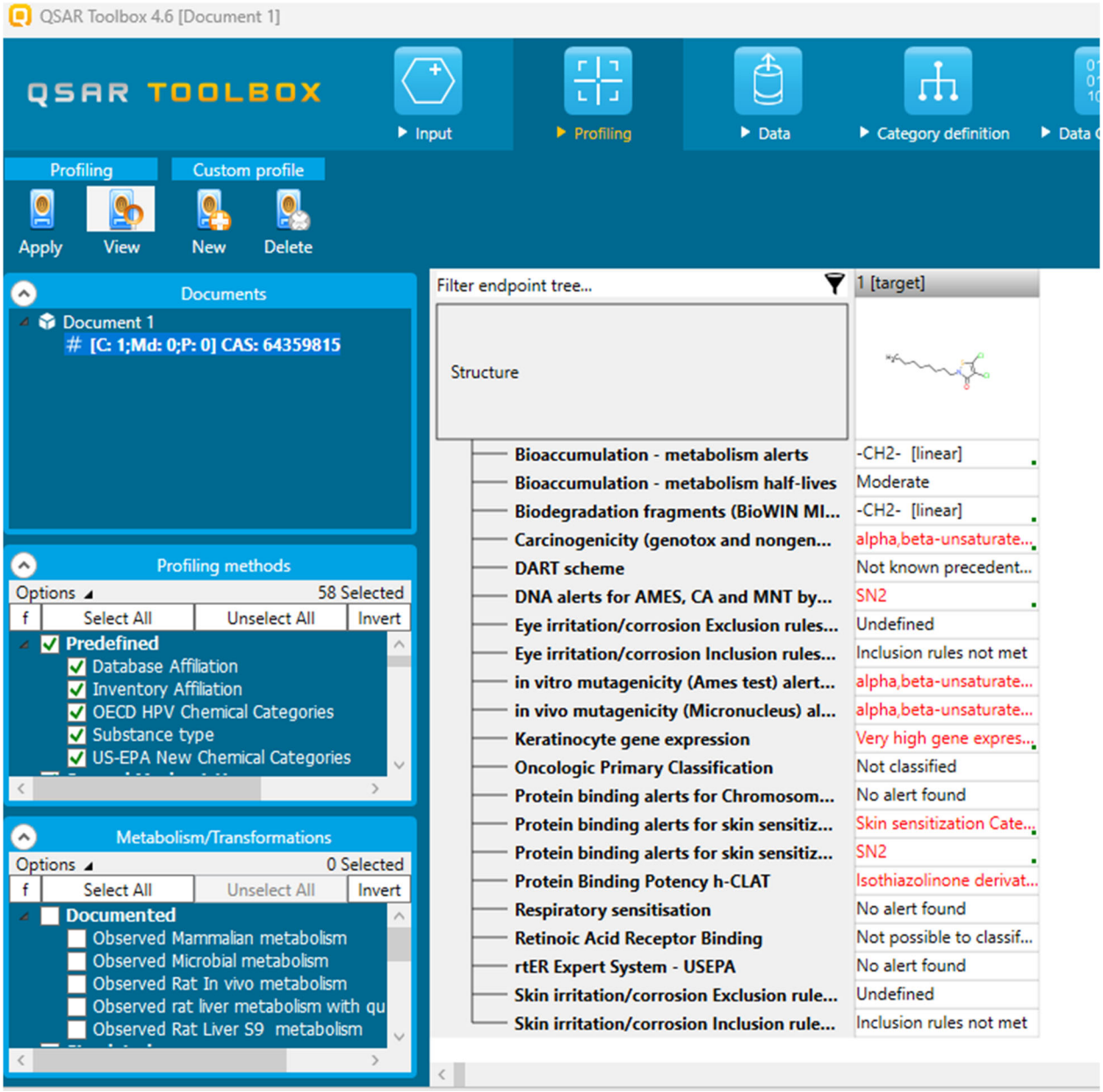
Download Lists

| ENDPOINT | HAZARD LEVEL | GREENSCREEN® | LIST NAME | HAZARD DESCRIPTION | OTHER LISTS |
|---|---|---|---|---|---|
| Endocrine Activity | H-M | LT-P1 | TEDX - Potential Endocrine Disruptors | Potential Endocrine Disruptor | |
| Acute Mammalian Toxicity | vH | LT-UNK | EU - GHS (H-Statements) Annex 6 Table 3-1 | H330 - Fatal if inhaled [Acute toxicity (inhalation) - Category 1 or 2] | +9 |
| | M | LT-UNK | EU - GHS (H-Statements) Annex 6 Table 3-1 | H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4] | |
| | vH | LT-UNK | GHS - New Zealand | Acute inhalation toxicity category 2 | |
| | vH | LT-UNK | GHS - Australia | H330 - Fatal if inhaled [Acute toxicity (inhalation) - Category 1 or 2] | |

| | | | | | |
|---|---|---|---|---|---|
| | vH | LT-UNK | GHS - Korea | H330 - Fatal if inhaled [Acute toxicity (inhalation) - Category 2] | |
| | H | LT-UNK | GHS - Korea | H311 - Toxic in contact with skin [Acute toxicity (dermal) - Category 3] | |
| | M | LT-UNK | GHS - Australia | H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4] | |
| | M | LT-UNK | GHS - Korea | H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4] | |
| | M | LT-UNK | GHS - New Zealand | Acute oral toxicity category 4 | |
| | pC | NoGS | US EPA - OPP - Registered Pesticides | FIFRA Registered Pesticide | |
| Skin Sensitization | H-M | LT-UNK | EU - GHS (H-Statements) Annex 6 Table 3-1 | H317 - May cause an allergic skin reaction [Skin sensitization - Category 1] | +3 |
| | H-M | LT-UNK | GHS - Korea | H317 - May cause an allergic skin reaction [Skin sensitization - Category 1] | |
| | H | LT-UNK | GHS - New Zealand | Skin sensitisation category 1 | |
| | H-M | LT-UNK | GHS - Australia | H317 - May cause an allergic skin reaction [Skin sensitization - Category 1] | |
| Skin Irritation/Corrosivity | vH | LT-UNK | EU - GHS (H-Statements) Annex 6 Table 3-1 | H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1A or 1B or 1C] | +4 |
| | vH | LT-UNK | GHS - Australia | H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1A or 1B or 1C] | |
| | vH | LT-UNK | GHS - Korea | H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1] | |
| | vH | LT-UNK | GHS - New Zealand | Skin corrosion category 1C | |
| | pC | NoGS | DK-EPA - Danish Advisory List | Skin Irrit. 2 - Causes skin irritation (modeled) | |
| Eye Irritation/Corrosivity | vH | LT-UNK | EU - GHS (H-Statements) Annex 6 Table 3-1 | H318 - Causes serious eye damage [Serious eye damage/eye irritation - Category 1] | +1 |
| | vH | LT-UNK | GHS - New Zealand | Serious eye damage category 1 | |

| | | | | | |
|---|---|---|---|---|---|
| Acute Aquatic Toxicity | vH | LT-UNK | EU - GHS (H-Statements) Annex 6 Table 3-1 | H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1] | +3 |
| | vH | LT-UNK | GHS - Korea | H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1] | |
| | pC | NoGS | DK-EPA - Danish Advisory List | Aquatic Acute1 - Very toxic to aquatic life (modeled) | |
| | pC | NoGS | DK-EPA - Danish Advisory List | Aquatic Chronic1 - Very toxic to aquatic life with long lasting effects (modeled) | |
| T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)] | vH | LT-UNK | GHS - New Zealand | Hazardous to the aquatic environment - acute category 1 | +3 |
| | vH | LT-P1 | GHS - New Zealand | Hazardous to the aquatic environment - chronic category 1 | |
| | U | LT-P1 | EU - GHS (H-Statements) Annex 6 Table 3-1 | H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1] | |
| | U | LT-P1 | GHS - Australia | H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1] | |
| Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation | U | LT-P1 | German FEA - Substances Hazardous to Waters | Class 3 - Severe Hazard to Waters | |

## APPENDIX D: OECD Toolbox Respiratory Sensitization Results for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)



## APPENDIX E: EPI Suite™ Modeling Results for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (CAS #64359-81-5)

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

CAS Number: 64359-81-5
SMILES : O=C1C(CL)=C(CL)SN1CCCCCCCC
CHEM : 3(2H)-Isothiazolone, 4,5-dichloro-2-octyl-
MOL FOR: C11 H17 CL2 N1 O1 S1
MOL WT : 282.23
---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): 4.40
Boiling Point (deg C) : ------
Melting Point (deg C) : 410.00
Vapor Pressure (mm Hg) : 0.00098
Water Solubility (mg/L): 3.1
Henry LC (atm-m3/mole) : 2.07E-006

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 3.59

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 375.31 (Adapted Stein & Brown method)
Melting Pt (deg C): 140.03 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 1.03E-009 (Modified Grain method)
VP (Pa, 25 deg C) : 1.37E-007 (Modified Grain method)
Subcooled liquid VP: 6.29 mm Hg (-999 deg C, user-entered VP )
: 839 Pa (-999 deg C, user-entered VP )

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 0.0031
log Kow used: 4.40 (user entered)
melt pt used: 410.00 deg C

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 27.449 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Thiazolones (Iso-)
Vinyl/Allyl Halides

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 1.86E-007 atm-m3/mole (1.89E-002 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: 2.070E-006 atm-m3/mole (2.097E-001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:

HLC: 1.174E-004 atm-m3/mole (1.190E+001 Pa-m3/mole)
VP: 0.00098 mm Hg (source: User-Entered)
WS: 3.1 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 4.40 (user entered)
Log Kaw used: -4.072 (user entered)
Log Koa (KOAWIN v1.10 estimate): 8.472
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.4988
Biowin2 (Non-Linear Model) : 0.0541
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.5275 (weeks-months)
Biowin4 (Primary Survey Model) : 3.5084 (days-weeks )
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.3628
Biowin6 (MITI Non-Linear Model): 0.0677
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.5914
Ready Biodegradability Prediction: NO

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

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 839 Pa (6.29 mm Hg)
Log Koa (Koawin est ): 8.472
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 3.58E-009
Octanol/air (Koa) model: 7.28E-005
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 1.29E-007
Mackay model : 2.86E-007
Octanol/air (Koa) model: 0.00579

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 29.7894 E-12 cm3/molecule-sec
Half-Life = 0.359 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 4.309 Hrs
Ozone Reaction:
OVERALL Ozone Rate Constant = 0.003579 E-17 cm3/molecule-sec
Half-Life = 320.239 Days (at 7E11 mol/cm3)
Fraction sorbed to airborne particulates (phi):
2.08E-007 (Junge-Pankow, Mackay avg)
0.00579 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 2262 L/kg (MCI method)
Log Koc: 3.355 (MCI method)
Koc : 2154 L/kg (Kow method)
Log Koc: 3.333 (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 = 1.196 (BCF = 15.7 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.2616 days (HL = 1.826 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.771 (BCF = 590.4)
Log BAF Arnot-Gobas method (upper trophic) = 2.773 (BAF = 592.9)
log Kow used: 4.40 (user entered)

Volatilization from Water:
Henry LC: 2.07E-006 atm-m3/mole (entered by user)
Half-Life from Model River: 476.9 hours (19.87 days)
Half-Life from Model Lake : 5343 hours (222.6 days)

Removal In Wastewater Treatment:
Total removal: 50.72 percent
Total biodegradation: 0.48 percent
Total sludge adsorption: 50.18 percent
Total to Air: 0.06 percent
(using 10000 hr Bio P,A,S)

**Level III Fugacity Model: (MCI Method)**

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| **Air** | **0.339** | **8.61** | **1000** |
| **Water** | **17.4** | **900** | **1000** |
| **Soil** | **80** | **1.8e+003** | **1000** |
| **Sediment** | **2.24** | **8.1e+003** | **0** |

**Persistence Time: 1.08e+003 hr**

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

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.339 | 8.61 | 1000 |
| Water | 17.4 | 900 | 1000 |
| water | (17.3) | | |
| biota | (0.0218) | | |
| suspended sediment | (0.0588) | | |
| Soil | 80 | 1.8e+003 | 1000 |
| Sediment | 2.24 | 8.1e+003 | 0 |

Persistence Time: 1.08e+003 hr

Level III Fugacity Model: (EQC Default)

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.313 | 8.61 | 1000 |
| Water | 15.6 | 900 | 1000 |
| water | (15.3) | | |
| biota | (0.0192) | | |
| suspended sediment | (0.236) | | |
| Soil | 74.9 | 1.8e+003 | 1000 |
| Sediment | 9.28 | 8.1e+003 | 0 |
| Persistence Time: | 1.17e+003 hr | | |

## APPENDIX F: Change in Benchmark Score

Table 6 provides a summary of changes to ToxServices' GreenScreen® Benchmark™ for 4,5-dichloro-2-octyl-2H-isothiazol-3-one. This is a new GreenScreen® assessment.

**Table 6: Change in GreenScreen® Benchmark™ for 4,5-Dichloro-2-octyl-2H-isothiazol-3-one**

| Date | GreenScreen® Benchmark™ | GreenScreen® Version | Comment |
|---|---|---|---|
| August 29, 2023 | BM-2 | v. 1.4 | New assessment |
| October 16, 2023 | BM-2 | v. 1.4 | No change in BM score. ToxServices corrected a typographical error in the Systemic Toxicity (repeated dose) section. |

**Licensed GreenScreen® Profilers**

**4,5-Dichloro-2-octyl-2H-isothiazol-3-one GreenScreen® Evaluation Prepared by:**

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Jennifer Rutkiewicz, Ph.D.
Senior Toxicologist
ToxServices LLC

**4,5-Dichloro-2-octyl-2H-isothiazol-3-one GreenScreen® Evaluation QC'd by:**

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