GreenScreen® Chemical Assessment

[Cyrene (53716-82-8)]

Method Version: GreenScreen® Version 1.4[1]

Assessment Details[2]:

Assessment Type:	Certified
Assessment Prepared By:	WAP Sustainability
Assessment Prepared For:	WA Ecology
Date Assessment Completed:	2/31/2023
Assessment Expiration Date:	2/31/2026
Assessor Type: (Licensed GreenScreen Profiler or equivalent, Authorized GreenScreen Practitioner or Unaccredited)	Licensed GreenScreen® Profiler

[1] Use GreenScreen® Chemical Hazard Assessment Guidance (Guidance) v1.4 in Section I
[2] **Assessment Type**: GreenScreen reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen Practitioner), or "CERTIFIED" (by Licensed GreenScreen Profiler or equivalent).
Assessment Prepared By: Licensed GreenScreen Profilers must provide name of organization; Authorized GreenScreen Practitioners must provide their name.
Assessment Prepared For: Optional for Licensed GreenScreen Profilers, mandatory for Authorized Practitioners.
Date Assessment Completed: Assessments by Licensed GreenScreen Profilers require quality control tracked via internal documentation.
Assessment Expiration Date: Assessments expire three years from the date of completion.

GREENSCREEN BENCHMARK™ SUMMARY:

This chemical assessment report includes a GreenScreen Benchmark™ score and results for Cyrene (53716-82-8) only.

No marketing claims can be made without licensing through Clean Production Action.

GreenScreen Benchmark Score:
Cyrene (53716-82-8) was assigned a GreenScreen® Benchmark Score of 3 ("Use but Still Opportunity for Improvement") as it has Moderate toxicity for Group II endpoints (i.e., Systemic Toxicity/Organ Effects-Single Exposure and eye irritations) This corresponds to GreenScreen® Benchmark criteria 3c in CPA 2015. Data gaps (DG) exist for endocrine activity (E) and respiratory sensitization (SnR). As outlined in CPA (2015) Section 13.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), Cyrene meets requirements for a GreenScreen® Benchmark Score of 3 despite the hazard data gaps. In a worst-case scenario, if Cyrene were assigned a High score for respiratory sensitization, it would be assigned a score of Benchmark 2.

HAZARD CLASSIFICATION SUMMARY

Table 1. GreenScreen Hazard Summary Table:[3,4]

GreenScreen Hazard Summary Table for Cyrene (53716-82-8)																			
Group I Human					**Group II and II* Human**									**Ecotox**		**Fate**		**Physical**	
Carcinogenicity	**Genotoxicity/Mutagenicity**	**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**
						single	repeat*	single	repeat*	*	*								
L	**L**	**L**	*L*	DG	**L**	**M**	**L**	*L*	**L**	**L**	DG	**L**	**M**	**L**	*L*	**vL**	**vL**	**L**	**L**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that Group II Human Health endpoints 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.

[3] See Appendix A for a glossary of hazard endpoint acronyms.
[4] See Appendix B for alternative GreenScreen Hazard Summary Table (Classification presented by exposure route). If such summaries are presented, they must be included in addition to the Hazard Summary Table above and placed in an Appendix to the report.

SCOPE OF ASSESSMENT

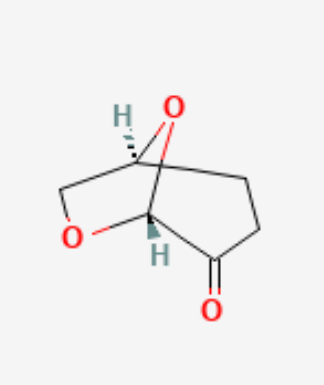
Chemical Name (CASRN): Cyrene (53716-82-8)

Also Called (List Synonyms):

PubChem 2023
53716-82-8
Dihydrolevoglucosenone
(1S,5R)-6,8-dioxabicyclo[3.2.1]octan-4-one
(1S,5R)-6,8-Dioxabicyclo(3.2.1)octan-4-one
(1beta,5beta)-6,8-Dioxabicyclo[3.2.1]octane-4-one

Chemical Structure:

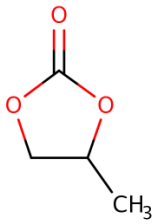
PubChem 2023



Suitable analogs or moieties of chemicals used in this assessment (CASRN(s)):
No analogs were located using the QSAR Toolbox

The US EPA Genra tool and the US EPA EPA CompTox Chemicals Dashboard were used to identify available analogs for the compound. The GenRA approach was developed using available chemistry descriptor information, bioactivity High Throughput Screening (HTS) data from the ToxCast program and in vivo toxicity data from ToxRefDB v.1.0. The read-across prediction is a similarity weighted activity of nearest neighbors (source chemicals) based on chemistry and/or bioactivity descriptors. Using the Morgan fingerprints method for identifying nearest neighbor propylene carbonate (CAS# 108-32-7) was identified based on the highest Jaccard similarity metric of 0.20 taking into account chemical features. This analog was used to help address the carcinogenicity endpoint.

Chemical Structure(s) of suitable analog(s) and/or moieties:



Propylene carbonate (CAS# 108-32-7)

For Inorganic Chemicals and relevant particulate organics (*if not relevant, list NA*) Define Properties:

ECHA 2023a

1. Particle size (e.g., silica of respirable size): The granulometry study does not need to be conducted as the substance is marketed and used in a non-solid form (liquid).
2. Structure (e.g., amorphous vs. crystalline): liquid
3. Mobility (e.g., water solubility, volatility): Water solubility in the range 52.6 to 90.0 w/w% at 20.0 ± 0.5°C and pH 3.09 - 3.15 was determined for Cyrene using a flask method with visual observation.
4. Bioavailability: NA

Identify potential applications/functional uses of the chemical:

Milescu 2021

- Cyrene has been explored as a bio-based solvent in wire coatings, filtration membranes industry, pharmaceuticals, graphene dispersion, cross-coupling, polymers, MOFs syntheses, solvent extraction or drug delivery.

Table 2. Environmental Transformation Products 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
	Aerobic biotransformation	Hydroxyglutarate	NA	Y	Y	NoGSLT
	Aerobic biotransformation	(2Z,4Z)-2,5-Dihydroxy-2,4-hexadienedioic acid	85844 0-64-9	Y	Y	NoGSLT
End of Life	Ultimate biodegradation	CO2	124-38-9	Y	N	LT-UNK
End of Life	Ultimate biodegradation	H20	7732-18-5	Y	N	BM4

Report rationale for each determination as to whether an identified environmental transformation product is feasible and relevant:

A biodegradation value of 99% in 28 days (DOC removal) was determined for the substance in accordance with OECD Test Guideline 301A and in compliance with GLP. However, no information was located regarding the structure of the breakdown products. Therefore, the Canonical smiles for the compound (C1CC(=O)C2OCC1O2; Pubchem 2023) was entered into the EAWAG pathway prediction system. The likely aerobic biotransformation pathway was selected. It is likely that the two metabolites predicted through aerobic biotransformation will continue to degrade. Based upon BOD and evolved CO^2 data, it is likely the initial biotransformation products will undergo rapid ultimate biodegradation to CO_2 'and H_20. These two ultimate biodegradation products are not considered relevant based on GreenScreen guidance (Section 14.3.2 Step 2; GreenScreen Method 2018).

HAZARD CLASSIFICATION SUMMARY[5,6]

GROUP I HUMAN HEALTH EFFECTS (GROUP I HUMAN)

Carcinogenicity (C): *L*

Cyrene (53716-82-8) was assigned a hazard classification level of Low for carcinogenicity based on no structural alerts for CMR in the OECD QSAR. In addition, a dermal carcinogenicity study using the analog propylene carbonate did not demonstrate tumor formation. The hazard conclusion is based on structural alerts and a dermal study for an analog compound and is therefore reported with low confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023b

- The carcinogenic potential of propylene carbonate was applied to the dorsal skin of mice two times a week for up to 104 weeks at a dose of 50 µL/application of a 100% concentration. The only statistically significant differences pointed out in the study report between the untreated mice and the treated mice was a reduction in body weight for the rats treated with the test substance. The treated skin remained clinically normal for the majority of mice in the propylene carbonate group and no treatment area skin tumors were seen in any of the mice. No NOAEL was identified as the only tested dose did no demonstrate tumor formation.

- Estimated Data: None

[5] Include computer-modeling outputs in Appendix C.
[6] References may be provided under each hazard endpoint or at the end of document.

- No QSAR alerts were identified for CMR in the OECD QSAR.

Mutagenicity/Genotoxicity (M): L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for Mutagenicity/Genotoxicity based on negative results reported from *in vitro* assays using the chemical of interest. The low hazard conclusion is based on high quality studies reported for the chemical of interest therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- Cyrene has been tested in a valid bacterial reverse mutation assay, conducted according to OECD Test Guideline 471 (1997) and in compliance with GLP, using Salmonella typhimurium strains S. typhimurium TA 1535, TA 1537, TA 98, TA 100, and TA 102. No increase in the number of revertants was observed in any test strain, with or without metabolic activation when tested up to limit concentration. Appropriate positive, negative, and solvent controls were added and gave expected results. It is concluded that the test substance is negative for mutagenicity to bacteria under the conditions of the test.
- Cyrene has been tested for ability to cause chromosome aberrations in cultured peripheral human lymphocytes in an in vitro cytogenicity study, conducted according to OECD Test Guideline 487 and in compliance with GLP. No increase in the number of cells with aberrations was observed either with or without metabolic activation in cultured peripheral human lymphocytes. Appropriate solvent and positive controls were included and gave expected results. It is concluded that the test substance is negative for the induction of chromosome aberrations under the conditions of this study.
- Cyrene has been tested in a valid in vitro mammalian mutagenicity study, conducted according to OECD Test Guideline 476 and in compliance with GLP, using mouse lymphoma L5178Y cells. Cyrene did not induce mutation at the HPRT locus of L5178Y mouse lymphoma cells when tested for 3 hours up to cytotoxic concentrations in the absence of a metabolic activation system (S9) and up to a concentration equivalent to 10 mM in the presence of metabolic activation system. Appropriate solvent, negative and positive controls were included and gave expected results. It is concluded that the test substance is negative for mutagenicity to mammalian cells under the conditions of this study.

- Estimated Data: None

Reproductive Toxicity (R): L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for reproductive toxicity based on the absence of reproductive effects observed in animals exposed to the 1,000 mg/kg-bw Cyrene. This hazard conclusion was based on a guideline study conducted in compliance with GLP using the chemical of interest and therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, conducted according to OECD Test Guideline 422 and in compliance with GLP, daily oral gavage administration of 0, 30, 300, or 1,000 mg/kg bw/day Cyrene to male rats for 42 consecutive days and to female rats for up to 55 days (pre-pairing, throughout gestation, and during the first 2 weeks of lactation) did not result in any systemic toxicity to adults. Cyrene related effects consisted of hematology, clinical chemistry, and organ weight changes following administration of 300 or 1,000 mg/kg bw/day. Daily vaginal lavage (washings) was conducted for all females during acclimation (pre-dose), from 1 week after arrival until the day prior to dosing. The stage of estrous was recorded, and only females with regular 4-to-5-day cycles were included on study. Daily vaginal lavage was conducted for females from the start of dosing until the confirmation of mating and on the morning of LD 14, prior to necropsy. Males were sacrificed on Study Day 43 (Post-Pairing Day 22). Females were sacrificed on LD 14 (those that achieved pregnancy) or Day 26 post coitum (those that did not litter). Animals were fasted overnight prior to sacrifice. Animals were sacrificed in a controlled randomization sequence, where possible, by isoflurane anesthesia. Once a suitable deep plane of anesthesia was established, major blood vessels were severed to exsanguinate the animal. After sacrifice, macroscopic examinations were conducted, and all lesions were recorded. The uterus of any apparently non pregnant female was immersed in a 10% ammonium sulfide solution to reveal any evidence of implantation. No effect on estrous cyclicity or sperm measures were noted for test article-treated animals, compared with controls. No test article-related effect on mating or fertility was noted. No toxicologically significant differences were evident in mean number of implantation sites or pups born. Post-implantation survival index for dams administered 1,000 mg/kg bw/day was 7% lower than controls; however, statistical significance was not achieved. This decrease in the post-implantation survival index was attributable to the total in utero litter loss in one high dose female which impacted the mean percentile post-implantation loss. Other live birth or survival indices were not affected. As such, the marginally lower implantation survival index was considered to have arisen incidentally. The effects were liver and enzyme changes that were considered to represent an adaptive response to

xenobiotic administration, and in the absence of any microscopic correlates the changes were considered not to represent an adverse effect. Therefore, based on the results of this study, the NOAEL for systemic toxicity was concluded to be greater than 1,000 mg/kg bw/day. The NOAEL for reproductive toxicity was concluded to be greater than 1,000 mg/kg bw/day.

- Estimated Data: None

Developmental Toxicity incl. Developmental Neurotoxicity (D): *L*

Cyrene (53716-82-8) was assigned a hazard classification level of Low for developmental toxicity based on the absence of developmental effects observed in animals exposed to the chemical of interest. While a NOAEL of 300 mg/kg-day is reported for developmental toxicity based on decreased pup birth weight on PND 4, this assessment concluded that this effect was not of toxicological significance and did not fulfill the classification of the substance as a GHS Category 2 reproductive toxicant. This hazard conclusion was based on a guideline study conducted in compliance with GLP using the chemical of interest. However, based on the discounting of the lower pups weights the hazard score is reported with low confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, conducted according to OECD Test Guideline 422 and in compliance with GLP, sub-acute oral gavage administration of 0, 30, 300, or 1,000 mg/kg bw/day Cyrene to female rats for 2 weeks prior to mating, during mating, throughout gestation, and up to lactation day 13 did not result in any systemic toxicity to maternal animals. Mean body weights for pups from litters of dams administered 1,000 mg/kg bw/day were not statistically significantly lower (approximately 8%) than the controls on post-natal day (PND) 1, but by post-natal day 4 the pup mean body weights were still low when compared to controls and reached statistical significance ($P < 0.01$; approximately 13%). Therefore, the maternal systemic NOAEL was concluded to be greater than 1,000 mg/kg bw/day, while the developmental NOAEL was concluded to be 300 mg/kg bw/day. Sex ratio (% males) was slightly higher for litters of dams administered 1,000 mg/kg bw/day, compared with controls. No statistical significance was achieved, as such, this slight increase in male sex ratio is not considered to be related to the test article.

- Estimated Data: None

Endocrine Activity (E): DG

Cyrene (53716-82-8) was assigned a hazard classification level of data gap for endocrine activity based on a lack of data for all endocrine pathways, notably estrogenicity. Data for other endocrine pathways shows the absence of overt differences in T4 or TSH levels for exposed pups, no nipples/areolae were present for male offspring from all litters, and no effect on ano-genital distance following dam exposure to Cyrene. The data for assessing potential hazard conclusions for available pathways is based on measured data for the chemical analog, however only some endocrine endpoints were measured. Thus, the hazard score is reported as a data gap.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, conducted according to OECD Test Guideline 422 and in compliance with GLP, sub-acute oral gavage administration of 0, 30, 300, or 1,000 mg/kg bw/day Cyrene to female rats for 2 weeks prior to mating, during mating, throughout gestation, and up to lactation day 13 did not result in any systemic toxicity to maternal animals. No overt differences in T4 or TSH levels were noted for pups exposed to Cyrene, compared with controls. No nipples/areolae were present for male offspring from all litters, including controls. Ano-genital distance was not affected following dam exposure to Cyrene.

- Estimated Data: None

GROUP II AND II* HUMAN HEALTH EFFECTS (GROUP II AND II* HUMAN)

Note: Group II and Group II endpoints are distinguished in the v1.4 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): L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for acute mammalian toxicity based on based on an oral LD50 values greater than 2,000 mg/kg and an inhalation LC50 greater than 5 mg/L. This hazard conclusion was based on guideline studies conducted in compliance with GLP using the chemical of interest and therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening:*
    - *DK-EPA - Danish Advisory List Acute Tox. 4 - Harmful if swallowed (modeled)*

- Measured Data

ECHA 2023a

- In the acute oral toxicity study, conducted according to OECD Test Guideline 423 and in compliance with GLP, the LD50 value for female rats was determined to be greater than 2,000 mg/kg bw. There were no mortalities, clinical signs of toxicity or adverse necropsy findings.

- In the acute inhalation toxicity study, conducted according to OECD Test Guideline 436 and in compliance with GLP, the concluded LC50 value was greater than 5.16 mg/L following 4-hour nose only exposure of rat to Cyrene aerosol at limit concentration.

- Estimated Data: None

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single): M

Cyrene (53716-82-8) was assigned a hazard classification level of Moderate for single dose systemic toxicity/organ effects based on minor toxicological effects reported following inhalation exposures. No mortalities, clinical signs of toxicity or adverse necropsy findings were noted following oral exposures. However, effects on the lung following inhalation exposures at 5.16 mg/L fulfill a GHS Category 3 (equivalent to GS Moderate) classification for respiratory tract irritation. The hazard score is based on study data and therefore is reported as high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the acute oral toxicity study, conducted according to OECD Test Guideline 423 and in compliance with GLP, the LD50 value for female rats was determined to be greater than 2,000 mg/kg bw. There was a 14-day observation period duration following administration: Specifically clinical observation was conducted 30 min and 1, 2, 3 and 5 h after treatment on test day 1; once daily during test days 2 - 15; observation of body weight: on test day 1 (prior treatment), 8 and 15 There were no

mortalities, clinical signs of toxicity or adverse necropsy findings.

- In the acute inhalation toxicity study, conducted according to OECD Test Guideline 436 and in compliance with GLP, the concluded LC50 value was greater than 5.16 mg/L following 4-hour nose only exposure of rat to Cyrene aerosol at limit concentration. There was a 14-day observation period duration following administration: Specifically, individual body weights were recorded on arrival, prior to treatment on the day of exposure (Day 0) and on Days 1, 3, 7 (limit test only) and at the end of the observation period. Other examinations performed included clinical signs at 1 hour after termination of exposure and subsequently once daily for up to 14 days. All animals, including the one that died, were subjected to a full external and internal examination and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity. The following macroscopic abnormalities were detected at necropsy of the animal found dead after 120 minutes exposure: Lungs – Abnormally red, dark patches. The following macroscopic abnormalities were detected at necropsy of animals surviving to the end of the observation period: Lungs – Pale, abnormally red, dark patches, dark red patches Kidneys – Pale. The observed abnormalities were considered likely to be due to local toxicity.

- Estimated Data: None

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*): L

Cyrene (53716-82-8) was assigned a hazard classification level Low for Repeat Dose Systemic Toxicity/Organ Effects including Immunotoxicity based on no effects reported following oral exposures. Specifically, no toxicity was observed following rat oral exposures up to 1,000 mg/kg-day. While the dosing was only for 55 days rather than the standard 90-day duration, adjusting the threshold criteria based on Haber's law would not change the hazard score (i.e., 55/90 x 1000 mg/kg = 611 mg/kg, which is > 100 mg/kg GreenScreen threshold)). The hazard score is based on high quality study data and therefore is reported as high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the key Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test with Cyrene, conducted according to OECD Test Guideline 422 and in compliance with GLP, a daily oral gavage administration of 0, 30, 300, or 1,000 mg/kg bw/day Cyrene to male rats for 42 consecutive days and to female rats for up to 55 days (pre-pairing, throughout gestation, and during the first 2 weeks of lactation) did not result in any systemic toxicity to adult animals.

Cyrene-related effects consisted of hematology, clinical chemistry, and organ weight changes following administration of 300 or 1,000 mg/kg bw/day. These effects were liver and enzyme changes that were considered to represent an adaptive response to xenobiotic administration, and in the absence of any microscopic correlates the changes were considered not to represent an adverse effect. No effects in thyroid hormone levels were noted for either sex administered the test item when compared with controls. Therefore, based on the results of this study, the NOAEL for systemic toxicity was concluded to be at least 1,000 mg/kg bw/day based on no adverse systemic effects.

- In the 14-day dose range finding study, a daily oral gavage administration of 250, 500, or 1,000 mg/kg bw/day Cyrene to rats over 14 consecutive days was well-tolerated. Based on this study, dose levels of 0, 30, 300, and 1,000 mg/kg bw/day were recommended as suitable dose levels for a subsequent combined repeated-dose toxicity study with a reproduction /developmental toxicity screening test (OECD Test Guideline 422).

- Estimated Data: None

Neurotoxicity (N-single): *L*

Cyrene (53716-82-8) was assigned a hazard classification level of Low for single exposure neurotoxicity based on no behavioral or clinical observations following single oral or inhalation exposures at 2,000 mg/kg-bw and 5.16 mg/L, respectively. Neurological conclusions are made based on clinical observation and behavior. The studies were not designed to specifically address neurological endpoints. Therefore, the hazard score is reported with low confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the acute oral toxicity study, conducted according to OECD Test Guideline 423 and in compliance with GLP, the LD50 value for female rats was determined to be greater than 2,000 mg/kg bw. There was a 14-day observation period duration following administration: Specifically clinical observation was conducted 30 min and 1, 2, 3 and 5 h after treatment on test day 1; once daily during test days 2 - 15; observation of body weight: on test day 1 (prior treatment), 8 and 15. There were no mortalities, clinical signs of toxicity or adverse necropsy findings.

- In the acute inhalation toxicity study, conducted according to OECD Test Guideline 436 and in compliance with GLP, the concluded LC50 value was greater than 5.16 mg/L following 4-hour nose only exposure of rat to Cyrene aerosol at limit concentration. There was a 14-day observation period duration following

administration: Specifically, individual body weights were recorded on arrival, prior to treatment on the day of exposure (Day 0) and on Days 1, 3, 7 (limit test only) and at the end of the observation period. Other examinations performed included clinical signs at 1 hour after termination of exposure and subsequently once daily for up to 14 days. All animals, including the one that died, were subjected to a full external and internal examination and any macroscopic abnormalities were recorded. No behavioral and clinical abnormalities were reported.

- Estimated Data: None

Neurotoxicity (N-repeated) (Group II*) L

Cyrene (53716-82-8) was assigned a hazard classification level of Low based on no locomotive effects in clinical observations and no effect reported in a Functional Observational Battery following oral exposures. Specifically, no toxicity was observed following rat oral exposures up to 1,000 mg/kg-day. While the dosing was only for 55 days rather than the standard 90-day duration, adjusting the threshold criteria based on Haber's law would not change the hazard score (i.e., 55/90 x 1000 mg/kg = 611 mg/kg, which is > 100 mg/kg GreenScreen threshold) The hazard score is based on high quality study data which included specific neurological observations and therefore is reported as high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the key Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test with Cyrene, conducted according to OECD Test Guideline 422 and in compliance with GLP, a daily oral gavage administration of 0, 30, 300, or 1,000 mg/kg bw/day Cyrene to male rats for 42 consecutive days and to female rats for up to 55 days (pre-pairing, throughout gestation, and during the first 2 weeks of lactation) did not result in any systemic toxicity to adult animals. Animals were observed at the beginning and end of the working day for signs of ill health or overt toxicity. Each animal was given a detailed physical examination once during acclimation then once daily from the start of dosing, including the day of necropsy. Functional Observational Battery (FOB assessments were performed to allow blind testing (in a manner so the observer did not know the dose group of animals during testing). Observations were performed at the same time on each occasion (approximately 2 hours post-dose), where possible. Locomotor activity was assessed in an automated photocell activity recorder for 30 minutes and was undertaken for five selected animals/sex/group (five males with the highest identification numbers and the first five littered females/group). Assessments were performed during Week 6 of dosing (Post Pairing Day 16) for males and on LD 7 for females. Activity counts were recorded at 5-minute intervals. The following

parameters were determined: Total activity counts; Total rears; Total mobile counts. No effects were observed for Behavior (functional findings) or Neuropathological findings. Based on the results of this study, a NOAEL for neurological toxicity is estimated herein to be at least 1,000 mg/kg bw/day.

- Estimated Data

Skin Sensitization (SnS) (Group II*) L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for skin sensitization based on a negative result using the mouse local lymph node assay (LLNA). This hazard conclusion was based on a guideline study conducted in compliance with GLP using the chemical of interest and therefore reported with high confidence.

Data
- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a
- The key local lymph node assay, conducted according to OECD Test Guideline 429 and in compliance with GLP, the test substance, Cyrene, was concluded to be not sensitizing to skin based on Stimulation Index (SI) values of <3 when tested at 5, 10 or 25% concentration.

- Estimated Data: None

Respiratory Sensitization (SnR) (Group II*): DG

Cyrene (53716-82-8) was assigned a hazard classification level of data gap for respiratory sensitization based on lack of adequate studies. In addition, no data was found for the analog chemical (propylene glycol) which was identified for the compound.

Data
- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data: None Found

- Estimated Data: None

Skin Irritation/Corrosivity (IrS) L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for skin irritation/corrosivity based on a negative result using OECD Test Guideline 404. This hazard conclusion was based on a guideline study conducted in compliance with GLP using the chemical of interest and therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the in vivo skin irritation study, conducted according to OECD Test Guideline 404 and in compliance with GLP, the test material was concluded to be not irritating to skin. No evidence of skin irritation was observed during this study. At all reading time points all scores were "0". No corrosive effects were noted. No evidence of skin irritation was noted in any test animal following 3-minute, 1-hour or 4-hour application.

- Estimated Data: None

Eye Irritation/Corrosivity (IrE): M

Cyrene (53716-82-8) was assigned a hazard classification level of Moderate for eye irritation/corrosivity based on results from the Ocular Irritation Test which showed an IDE score of 13.50 when tested at concentration of 125 µL. These results are interpreted to fulfill Category 2B (mildly irritating to eyes) based on GHS criteria. This hazard conclusion was based on a guideline study conducted in compliance with GLP using the chemical of interest and therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- In the first in vitro eye irritation study, conducted according to OECD Test Guideline 437 and in compliance with GLP, the test substance, no conclusion on the eye irritation potential of Cyrene could be made even though there were no corrosive/severe irritant properties detected. Following single application of 750 µL of undiluted test material to 3 corneas for 10 min at 32 ± 1 °C and further two hours incubation after test item removal, the calculated mean IVIS was 36.37 (threshold for serious eye damage: IVIS ≥ 55) resulting in inconclusive prediction on the eye

irritation potential of the substance. No increase of opacity or permeability of the corneas were observed for the negative control. The measured IVIS value of 1.10 lies within the historical range of the IVIS for negative control (0.00 - 2.84). The positive control showed clear opacity and distinctive permeability of the corneas with mean IVIS score of 68.22 corresponding eye damage. Relative to the negative control, the test item caused an increase of the corneal opacity and the permeability.

- In the second in vitro eye irritation study, conducted according to the Ocular Irritation Test Method, Cyrene was concluded to be irritating to eyes. In the study Cyrene was evaluated to predict its potential to cause ocular irritation. The concentrations of sample applied to the reagent solution for analysis were 50, 75, 100 and 125 µL. The results indicated that the Cyrene is irritating to eyes based on an IDE score of 13.50 when tested at concentration of 125 µL. These results are interpreted to fulfill Category 2B (mildly irritating to eyes) based on GHS criteria.

- Estimated Data: None

ECOTOXICITY (ECOTOX)

Acute Aquatic Toxicity (AA): L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for acute aquatic toxicity based on measured data in fish, aquatic invertebrates and algae which show LC50/EC50's >100 mg/L. This hazard conclusion was based on guideline studies conducted in compliance with GLP using the chemical of interest and therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- Fish: LC50 (96 h): >100 mg/L; NOEC: ≥100 mg/L (based on mortality) (limit test) (exposure is predominantly to the Gem Diol form). OECD Guideline 203 (Fish, Acute Toxicity Test)

- Daphnia: EC50 (48 h): >100 mg/L; NOEC: ≥100 mg/L (based on mobility) (limit test) (exposure is predominantly to the Gem Diol form). OECD Guideline 202 (Daphnia sp. Acute Immobilization Test)

- Algae: EC50 (72 h): >100 mg/L; EC10: >100 mg/L, NOEC: ≥100 mg/L (based on growth rate) (highest concentration tested) (exposure is predominantly to the Gem Diol form). OECD Guideline 201 (Alga, Growth Inhibition Test)

- Estimated Data: None

Chronic Aquatic Toxicity (CA): *L*

Cyrene (53716-82-8) was assigned a hazard classification level of Low for chronic aquatic toxicity based on measured data in algae which show an EC50 and NOEC >100 mg/L based on growth rate. This hazard conclusion is supported by EcoSar modeled data for neutral organics which shows ChV values >100 mg/L for fish Daphnia and Green Algae. This hazard conclusion was based on modeled data for 2 of the 3 trophic levels (fish and aquatic invertebrates) and therefore reported with low confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- Algae: EC50 (72 h): >100 mg/L; EC10: >100 mg/L, NOEC: ≥100 mg/L (based on growth rate) (highest concentration tested) (exposure is predominantly to the Gem Diol form). OECD Guideline 201 (Alga, Growth Inhibition Test).

- Estimated Data

ECHA 2023a

ECOSAR - The relevant predicted values, using ECOSAR v2.0 for the prediction of the aquatic toxicity of neutral organics, are:

- Fish 30-day NOEC mg/L 6482 mg/L
- Daphnia 16-day NOEC 1575 mg/L
- Green Algae NOEC 985 mg/L

ENVIRONMENTAL FATE (FATE)

Persistence (P): vL

Cyrene (53716-82-8) was assigned a hazard classification level of very Low for persistence based on a biodegradation value of 99% in 28 days (DOC removal) was determined for the substance in accordance with OECD Test Guideline 301A and in compliance with GLP. The hazard score is based on results from a guideline study and therefore is reported as high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- A biodegradation value of 99% in 28 days (DOC removal) was determined for the substance in accordance with OECD Test Guideline 301A and in compliance with GLP. Specifically, degradation of the Test Item: In the test flasks, containing the test item Cyrene and activated sludge (inoculum), the initial mean concentration of dissolved organic carbon (DOC) of 28.7 mg/L on Day 0 rapidly decreased after Exposure Day 8, reaching 99% decrease of the initial value within 14 days of exposure. The pass level for ready biodegradability (70% removal of DOC in a 10-day window within a 28-day period) was reached. Degradation in the Toxicity Control: In the toxicity control, containing the test item (corresponding to 50.1% of total DOC), the reference item (corresponding to 49.9% of total DOC) and activated sludge (inoculum), the initial DOC concentration of 58.4 mg/L measured on Day 0 decreased to 0.5 mg/L on Day 14. Biodegradation amounted to 99% within 14 days of exposure. Thus, according to the test guidelines, the test item was not inhibitory to activated sludge at the tested concentration of 50.4 mg/L because degradation was > 35% within 14 days.

- Estimated Data

EpiSuite

Level III Fugacity Model:

	Mass Amount (%)	Half-Life (Hr)	Emissions (kg/hr)
Air	0.695	10.4	1000
Water	42.5	360	1000
Soil	56.8	720	1000
Sediment	0.0795	3.24e+3	0

Bioaccumulation (B): vL

Cyrene (53716-82-8) was assigned a hazard classification level of very Low for bioaccumulation based a measured log know value that is <4. The hazard conclusion is based on measured data for the chemical of interest and is therefore reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- When Cyrene™ is in aqueous solution, the keto group (C=O) gains water (H2O) to form the corresponding Gem Diol {(1S,5R)-6,8-dioxabicyclo[3.2.1]octane-4,4-diol}. An equilibrium is established rapidly (meaning that the reaction is rapidly reversible). The ratio of the two forms is dependent upon the amount of water present. At concentrations relevant for the environment (aquatic media) and in vivo, the Gem Diol form predominates. A Log Kow value of -1.52 at 22.0 ± 1.0°C and pH 5.18 to 5.24 was

determined for Cyrene/Gem Diol. Therefore, the submission substance has low potential for bioaccumulation.

- Estimated Data: None

PHYSICAL HAZARDS (PHYSICAL)

Reactivity (Rx): L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for reactivity based on the absence of chemical groups that are associated with explosivity or oxidizing properties. The hazard score is based on physical chemical properties of the compound and therefore is reported with high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- The explosive properties of the submission substance were assessed based on structural examination. The molecule has no chemical groups that are associated with explosive properties.

- On the basis of chemical structure, the submission substance is expected to be incapable of reacting exothermically with combustible materials therefore the oxidizing properties study is not needed.

- The flammability of the substance in contact with water was assessed in accordance with EU Test Method A.12 and in compliance with GLP. Under the conditions of the study, the submission substance was determined to be non-hazardous as no gases were evolved in any of the four steps of the study.

- Estimated Data: None

Flammability (F): L

Cyrene (53716-82-8) was assigned a hazard classification level of Low for flammability based on a flash point of 108°C and boiling point of 227°C. Therefore, this substance is not classified as flammable based on the GHS criteria. The hazard score is based on measured data and is therefore reported as high confidence.

Data

- Lists
  - *Authoritative: None*
  - *Screening: None*

- Measured Data

ECHA 2023a

- The flammability of the substance in contact with water was assessed in accordance with EU Test Method A.12 and in compliance with GLP. Under the conditions of the study, the submission substance was determined to be non-hazardous as no gases were evolved in any of the four steps of the study.

- There is no indication on the basis of chemical structure and experience in handling and use that the substance is pyrophoric (flammable in contact with air).

- Cyrene is not classified for flammability according to Regulation (EC) No. 1272/2008. This is on the basis of a measured flash point of 108°C at 1013 hPa and a measured boiling point of 227°C at 1007 hPa.

- Estimated Data: None

REFERENCES

(May be provided under each hazard endpoint or at the end of document)

ECHA 2023a. Registered Substances Factsheets. 6,8-Dioxabicyclo[3.2.1]octan-4-one, (1S,5R)-. Available at https://echa.europa.eu/registration-dossier/-/registered-dossier/16252/1/1

ECHA 2023b. Registered Substances Factsheets. Propylene carbonate. Available at https://echa.europa.eu/registration-dossier/-/registered-dossier/16088

GreenScreen Guidance 2018. Available at https://www.greenscreenchemicals.org/learn/guidance-and-method-documents-downloads.

Milescu 2021. Polymer Chemistry Applications of Cyrene and its Derivative Cygnet 0.0 as Safer Replacements for Polar Aprotic Solvents. ChemSusChem 2021, 14, 3367.

PubChem 2023. Compound Summary. (1S,5R)-6,8-dioxabicyclo[3.2.1]octan-4-one. Available at https://pubchem.ncbi.nlm.nih.gov/compound/10975499

APPENDIX A: HAZARD CLASSIFICATION ACRONYMS

(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 – OPTIONAL HAZARD SUMMARY TABLE

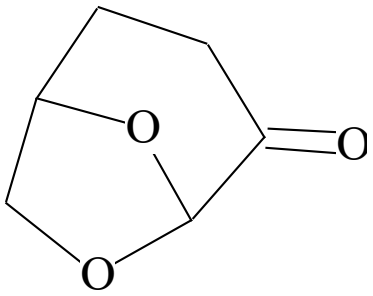
Exposure Route	GreenScreen Hazard Ratings: [*Chemical Name*]																	
	Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
	C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
							single	repeated*	single	repeated*										
oral																				
dermal																				
inhalation																				

APPENDIX C – MODELING RESULTS

Attach:

- **EPISuite Results for Cyrene (53716-82-8))**

EPI Suite Results for CAS




```
SMILES : O=C1C2OC(CC1)CO2
CHEM   :
MOL FOR: C6 H8 O3
MOL WT: 128.13
------------------------------ EPI SUMMARY (v4.11) --------------------------
Physical Property Inputs:
Log Kow (octanol-water):   ------
Boiling Point (deg C)  :   ------
Melting Point (deg C)  :   ------
Vapor Pressure (mm Hg) :   ------
Water Solubility (mg/L):   ------
Henry LC (atm-m3/mole) :   ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.68 estimate) = -0.28

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C):  203.43  (Adapted Stein & Brown method)
Melting Pt (deg C):  19.77  (Mean or Weighted MP)
VP(mm Hg,25 deg C):  0.439  (Mean VP of Antoine & Grain methods)
VP (Pa, 25 deg C) :  58.6  (Mean VP of Antoine & Grain methods)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L):  1.624e+005
log Kow used: -0.28 (estimated)
no-melting pt equation used
```

```
Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) =  1e+006 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method :   6.22E-008  atm-m3/mole  (6.30E-003 Pa-m3/mole)
Group Method:   Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC:  not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 4.557E-007 atm-m3/mole (4.618E-002 Pa-m3/mole)
VP:   0.439 mm Hg (source: MPBPVP)
WS:   1.62E+005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used:  -0.28  (KowWin est)
Log Kaw used:  -5.595  (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):  5.315
Log Koa (experimental database):  None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model)         :  -0.0013
Biowin2 (Non-Linear Model)     :   0.0022
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model):   2.8762  (weeks       )
Biowin4 (Primary Survey Model) :   3.6212  (days-weeks  )
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model)   :   0.5357
Biowin6 (MITI Non-Linear Model):  0.4742
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.3514
Ready Biodegradability Prediction:   YES

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

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled):  54 Pa (0.405 mm Hg)
Log Koa (Koawin est  ): 5.315
Kp (particle/gas partition coef. (m3/ug)):
Mackay model           :  5.56E-008
Octanol/air (Koa) model:  5.07E-008
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model    :  2.01E-006
Mackay model          :  4.44E-006
Octanol/air (Koa) model:  4.06E-006

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant =  24.6935 E-12 cm3/molecule-sec
Half-Life =     0.433 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =     5.198 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
```

```
Fraction sorbed to airborne particulates (phi):
3.23E-006 (Junge-Pankow, Mackay avg)
4.06E-006 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc    :  1.001  L/kg (MCI method)
Log Koc:  0.001       (MCI method)
Koc    :  6.09  L/kg (Kow method)
Log Koc:  0.785       (Kow method)

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

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.2169 days (HL = 0.06069 days)
Log BCF Arnot-Gobas method (upper trophic) = -0.030 (BCF = 0.9326)
Log BAF Arnot-Gobas method (upper trophic) = -0.030 (BAF = 0.9326)
log Kow used: -0.28 (estimated)

Volatilization from Water:
Henry LC:  6.22E-008 atm-m3/mole  (estimated by Bond SAR Method)
Half-Life from Model River: 1.066E+004  hours   (444 days)
Half-Life from Model Lake : 1.163E+005  hours   (4848 days)

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

Level III Fugacity Model:
Mass Amount    Half-Life    Emissions
(percent)        (hr)       (kg/hr)
Air       0.695           10.4         1000
Water     42.5            360          1000
Soil      56.8            720          1000
Sediment  0.0795          3.24e+003    0
Persistence Time: 431 hr
```

....

- **ECOSAR Results for Chemical Name (CASRN)**

ECOSAR Version 1.11 Results Page

SMILES : O=C1C2OC(CC1)CO2
CHEM :
CAS Num: 53716-82-8
ChemID1:

MOL FOR: C6 H8 O3
MOL WT : 128.13
Log Kow: -0.281 (EPISuite Kowwin v1.68 Estimate)
Log Kow: (User Entered)
Log Kow: (PhysProp DB exp value - for comparison only)
Melt Pt: (User Entered for Wat Sol estimate)
Melt Pt: (deg C, PhysProp DB exp value for Wat Sol estimate)
Wat Sol: 1.624E+005 (mg/L, EPISuite WSKowwin v1.43 Estimate)
Wat Sol: (User Entered)
Wat Sol: (PhysProp DB exp value)

Values used to Generate ECOSAR Profile

Log Kow: -0.281 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 1.624E+005 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics	: Fish	96-hr	LC50	11766.121
Neutral Organics	: Daphnid	48-hr	LC50	5412.191
Neutral Organics	: Green Algae	96-hr	EC50	1687.783
Neutral Organics	: Fish		ChV	897.122
Neutral Organics	: Daphnid		ChV	293.722
Neutral Organics	: Green Algae		ChV	276.529
Neutral Organics	: Fish (SW)	96-hr	LC50	14611.783
Neutral Organics	: Mysid	96-hr	LC50	50911.340
Neutral Organics	: Fish (SW)		ChV	393.944
Neutral Organics	: Mysid (SW)		ChV	8650.745
Neutral Organics	: Earthworm	14-day	LC50	384.031

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

Class Specific LogKow Cut-Offs

If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Neutral Organics:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50)
Maximum LogKow: 6.0 (Earthworm LC50)
Maximum LogKow: 6.4 (Green Algae EC50)
Maximum LogKow: 8.0 (ChV)

- **Other**