TRALOPYRIL

(CAS #122454-29-9)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: October 16, 2023

ToxServices Review Date: October 16, 2028[1]

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[1] Although CPA's Assessment Expiration Policy (CPA 2018a) indicates that Benchmark 1 assessments have no expiration date, ToxServices strives to review BM-1s in a five-year period to ensure currency of data presented in the BM-1 GreenScreen® assessments.

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GreenScreen® Executive Summary for Tralopyril (CAS #122454-29-9)

Tralopyril is a halogenated pyrrole biocide that acts by uncoupling mitochondrial oxidative phosphorylation, leading to cell dysfunction. It's commercially available under the trade name Econea™ and it is used mainly as an antifoulant in paints for commercial and recreational ships and other marine structures in fresh and salt water at use levels of 2.9% to 7.28%. Tralopyril is a heavy, white powder with low water solubility, low volatility, and low flammability.

Tralopyril was assigned a **GreenScreen Benchmark™ Score of 1** ("Avoid – Chemical of High Concern"). This score is based on the following hazard score combinations:

- Benchmark 1c
    - Very High Persistence-P + Very High Ecotoxicity (acute aquatic toxicity-AT, chronic aquatic toxicity-CA)
    - Very High P + Very High Group II Human (acute mammalian toxicity-AT, neurotoxicity-single exposure-Ns)
    - Very High P + High Group II* Human (repeated dose systemic toxicity STr*, repeated dose neurotoxicity-Nr*)
    - Very High P + High Group I Human (developmental toxicity-D)
- Benchmark 1e
    - High Group I Human (D)

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

ToxServices' GreenScreen® Benchmark Score for tralopyril has changed over time. ToxServices' original GreenScreen® assessment was performed in 2015 under version 1.2 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. In 2020, ToxServices changed the GreenScreen® benchmark score to a BM-1 due to reclassification of the developmental endpoint from Low (low confidence) to High (low confidence), reclassification of single exposure neurotoxicity endpoint from Data Gap to Very High (low confidence), and reclassification of persistence endpoint from High (low confidence) to Very High (low confidence) following a weight of evidence evaluation of this chemical's updated dataset. The BM-1 score is maintained with this 2023 version 1.4 update. In this update, the score for skin sensitization has changed from **L** (high confidence) to *L* (low confidence) based on re-evaluation of data and modeling. The scores for other endpoints remain the same.

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

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

Type I (input data) uncertainties in tralopyril's NAMs dataset include limited, or lack of, experimental data for carcinogenicity, endocrine activity, skin sensitization, respiratory sensitization, and persistence, and lack of established test methods for respiratory sensitization. Tralopyril's Type II (extrapolation output) uncertainties include lack of defined applicability domains of some modeling software

examining structural alerts, inability of OncoLogic to evaluate the entire structure of the target compound, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions and *in vitro* receptor binding activity assays, and the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization. Some of tralopyril's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for Tralopyril

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	**M**	**R**	**D**	**E**	**AT**	**ST**		**N**		**SnS**	**SnR**	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
						s	r*	s	r*	*	*								
M	**L**	*L*	*H*	DG	**vH**		**H**	*vH*	**H**	*L*	*L*	*M*	*M*	**vH**	**vH**	*vH*	**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 Tralopyril (CAS #122454-29-9)

Method Version: GreenScreen® Version 1.4
Assessment Type[2]: Certified
Assessor Type: Licensed GreenScreen® Profiler

GreenScreen® Assessment (v.1.2) Prepared By:
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Date: July 14, 2020

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Date: July 24, 2023; October 5, 2023

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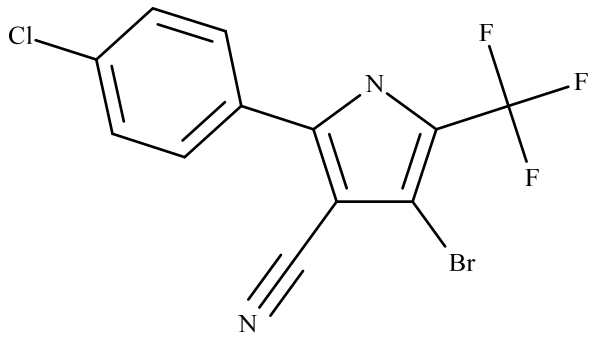
Date: August 29, 2023; October 16, 2023

ToxServices Review Date: October 16, 2028[3]

Chemical Name: Tralopyril

CAS Number: 122454-29-9

Chemical Structure(s):

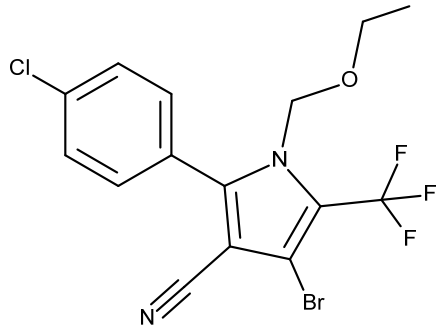


Tralopyril (CAS# 122454-29-9) (PubChem 2023)

[2] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[3] Although CPA's Assessment Expiration Policy (CPA 2018a) indicates that Benchmark 1 assessments have no expiration date, ToxServices strives to review BM-1s in a five-year period to ensure currency of data presented in the BM-1 GreenScreen® assessments.

Also called: 4-Bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)pyrrole-3-carbonitrile; UNII-MEC6MCY7QB; 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (PubChem 2023).

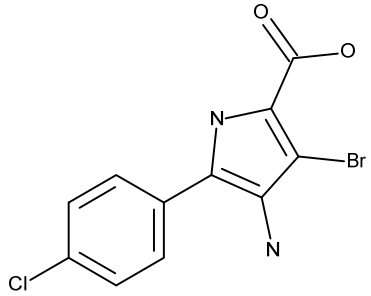
Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Tralopyril is a halogenated pyrrole resulting from the N-dealkylation of the ethoxymethyl group of chlorfenapyr (CAS #122453-73-0). Tralopyril is the active metabolite of the proinsecticide chlorfenapyr, which forms tralopyril in ratshrough mixed function oxidase-mediated cleavage of chlorfenapyr's N-ethoxymethyl sidechain. Chlorfenapyr has the following structure:



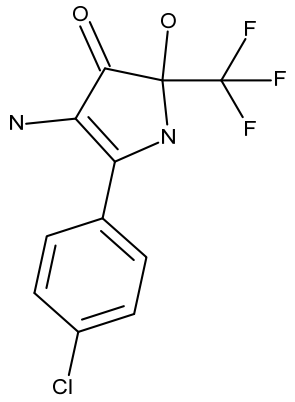
Chlorfenapyr (CAS #122453-73-0)

Tralopyril is a metabolite of chlorfenapyr (U.S. EPA 2014), and Tralopyril was shown to be the main phase I metabolite in a GLP-compliant *in vitro* study investigating the metabolism of chlorfenapyr in rats (Funk-Weyer and Kemper 2017). Therefore, ToxServices selected chlorfenapyr as a surrogate for tralopyril. However, chlorfenapyr has other metabolic pathways and the percent converted to tralopyril *in vivo* is not determined (JMPR 2018). A comparison of subchronic oral toxicity LOAELs reveals that tralopyril was 7 times more toxic than chlorfenapyr in rats. Based on comparison of maternal toxicity and developmental toxicity NOAELs and LOAELs, tralopyril is 15 and 45 times more toxic than chlorfenapyr for maternal and developmental toxicities, respectively. Tralopyril is also more neurotoxic than chlorfenapyr in repated dose oral and inhalation toxicity studies (JMPR 2013). Therefore, ToxServices considers chlorfenapyr to be a weak surrogate for tralopyril.

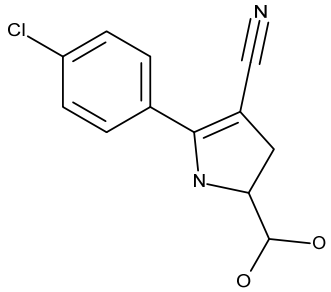
Additionally, the United States Environmental Protection Agency (U.S. EPA) identified three rapid degradation products of tralopyril as CCL322.250 (parent compound minus fluorines and remaining carbon hydrated); CL325,195 (the debrominated parent compound with hydration forming a ketone and an ortho hydroxylated trifluoromethyl group), and CL322,248 (the debrominated form of CL322,250) (U.S. EPA 2019). Therefore, ToxServices also used data on the three degradation products to support this evaluation. The structures of these three compounds are presented below.



CL322,250 (No CAS) (U.S. EPA 2019)



CL325,195 (CAS 122454-23-3) (U.S. EPA 2019)



CL322,248 (No CAS) (U.S. EPA 2019)

Identify Applications/Functional Uses:
Antifoulant in paint at use levels of 2.9% to 7.28% (U.S. EPA 2019)

Known Impurities:
No information is available. The screen is performed on the theoretical pure substance. According to the GreenScreen® Guidance, impurities present at < 100 ppm require a List Translator screening, while those present at > 100 ppm require separate full GreenScreen® evaluations. Impurities are not evaluated in this assessment. Instead, they can be evaluated at the product level, should they be present at > 100 ppm.

GreenScreen® Summary Rating for Tralopyril[4,5 6,7]: Tralopyril was assigned a **GreenScreen Benchmark™ Score of 1** ("Avoid – Chemical of High Concern") (CPA 2018b). This score is based on the following hazard score combinations:

- Benchmark 1c
    - Very High Persistence-P + Very High Ecotoxicity (acute aquatic toxicity-AT, chronic aquatic toxicity-CA)

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

  - Very High P + Very High Group II Human (acute mammalian toxicity-AT, neurotoxicity-single exposure-Ns)
  - Very High P + High Group II* Human (repeated dose systemic toxicity STr*, repeated dose neurotoxicity-Nr*)
  - Very High P + High Group I Human (developmental toxicity-D)
- Benchmark 1e
  - High Group I Human (D)

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

Figure 1: GreenScreen® Hazard Summary Table for Tralopyril

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
M	**L**	*L*	*H*	DG	**vH**		**H**	*vH*	**H**	*L*	*L*	*M*	*M*	**vH**	**vH**	*vH*	**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 of tralopyril is expected to be an important environmental fate process based on its hydrolysis half-lives of 14-175 days at pH 5 to 0.1-0.6 days at pH 9 and in seawater. CL 322,250, which is the defluorinated parent compound with a carboxylic acid group, and hydrogen fluoride, are persistent. Under anaerobic conditions such as in the bottom sediment, and in seawater, CL322,250 can further degrade to CL 322,248, which is the debromination product of CL322,250, and hydrogen bromide (U.S. EPA 2019). When heated to decomposition, tralopyril emits toxic fumes of carbon oxides, nitrogen oxides (NOx), hydrogen chloride gas, hydrogen bromide gas and hydrogen fluoride (U.S. EPA 2013).

The Pharos listings for the identified transformation products are tabulated below (Table 1). Some of the degradation products are known or possible Benchmark 1 chemicals. No CAS numbers or data were identified for the transformation products CL322,250 and CL 322,248. As tralopyril itself is a Benchmark 1 chemical, the hazards of the transformation products will not impact its final Benchmark score.

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[8,9]**
End of life	Heat decomposition, hydrolysis	Hydrogen bromide (HBr)	10035-10-6	Yes	Yes	LT-P1
End of life	Heat decomposition, hydrolysis	Hydrogen fluoride (HF)	7664-39-3	Yes	Yes	BM-1
End of Life	Heat decomposition	Nitrogen dioxide (NO_2)	10102-44-0	Yes	Yes	LT-P1
End of Life	Heat decomposition	Nitrogen trioxide (N_2O_3)	10544-73-7	Yes	Yes	LT-UNK
End of Life	Heat decomposition	Nitrogen tetroxide (N_2O_4)	10544-72-6	Yes	Yes	LT-UNK
End of Life	Heat decomposition	Nitrogen oxide (NO_x)	11104-93-1	Yes	Yes	LT-UNK
End of Life	Heat decomposition	Hydrogen chloride (HCl)	7647-01-0	Yes	Yes	BM-2
End of Life	Hydrolysis	CL322,250	None	Yes	Yes	None
End of Life	Hydrolysis	CL 322,248	None	Yes	Yes	None

Introduction

Tralopyril is a halogenated pyrrole biocide that acts by uncoupling mitochondrial oxidative phosphorylation, leading to cell dysfunction. Its trade name is Econea™ and it is used mainly as an antifoulant in commercial and recreational paint for ships and other marine structures in fresh and salt water at use levels of 2.9% to 7.28%. Tralopyril is added to paint to control barnacles, mussels, snails, tube worms, algae, slime, and other marine fouling organisms. Some antifouling paints include only tralopyril as the active ingredient, and some are co-formulated with zinc pyrithione, copper oxide, or 3(2H)-isothizolone, 4,5-dichloro-2-octyl (DCOIT) (U.S. EPA 2019).

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

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

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2023a). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

Tralopyril is not 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),[10] which are not considered GreenScreen® Specified Lists but are additional information

[8] 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.
[9] A GreenScreen® assessment of a transformation product depends on the Benchmark score of the parent chemical (see GreenScreen® Guidance).
[10] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

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 tralopyril can be found in Appendix C.

- According to Pharos, tralopyril was previously determined to be a Benchmark-2 chemical according to ToxServices' version 1.2 GreenScreen® from 2015, which is expired according to ToxServices' internal policy; therefore, the current GreenScreen® assessment brings it up to date. ToxServices performed additional updates to this document in 2020 (not publicly available), as shown in Appendix R.
- Tralopyril is not listed on the U.S. DOT list.
- Tralopyril is on the following lists for multiple endpoints.
    - GHS-Korea – Hazardous to the aquatic environment (chronic) – Category 1 – H410 – Very toxic to aquatic life with long lasting effects
    - GHS-Japan – Hazardous to the aquatic environment (chronic) – Category 1 – H410
    - GHS New Zealand – Hazardous to the aquatic environment (chronic) – Category 1
- GreenScreen®-specified lists that correspond to single endpoints are discussed in their respective hazard assessment section below.

Hazard Statement and Occupational Control

No Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for tralopyril; however, the majority of notifiers suggest classifications for acute and chronic aquatic toxicity as shown in Table 2.

Table 2: GHS H Statements for Tralopyril (CAS #122454-29-9) (ECHA 2023)

H Statement	H Statement Details
H400	Very toxic to aquatic life (Aquatic Acute 1)
H410	Very toxic to aquatic life with long lasting effects (Aquatic Chronic 1)

There are no regulatory occupational exposure limits specific to tralopyril, however manufacturer recommendations were identified as shown in Table 3.

Table 3: Occupational Exposure Limits and Recommended Personal Protective Equipment for Tralopyril (CAS #122454-29-9)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Respiratory: Dust/mist filtering respiratory (NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, P, R, or HE filter.	Janssen Pharmaceuticals, Inc. 2015	TWA 0.040 mg/m^3 (J&J OEL)	Janssen Pharmaceuticals, Inc. 2015
Hands: Gloves (nitrile rubber, natural rubber, butyl rubber, neoprene, polyethylene, PVC or Viton®).			
Eyes: respirator with full face mask.			
Skin and body: closed work clothing with long sleeves.			

NIOSH: National Institute for Occupational Safety and Health

Table 3: Occupational Exposure Limits and Recommended Personal Protective Equipment for Tralopyril (CAS #122454-29-9)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
OEL: Occupational Exposure Limit TWA: Time Weighted Average			

Physicochemical Properties of Tralopyril

Tralopyril is a heavy, white powder with low water solubility, low melting point, and low vapor pressure. Therefore, if released to the environment, it is likely to sorb to sediment and soil. The vapor pressure indicates that it is not volatile.

Table 4: Physical and Chemical Properties of Tralopyril (CAS #122454-29-9)		
Property	**Value**	**Reference**
Molecular formula	$C_{12}H_5BrClF_3N_2$	PubChem 2023
SMILES Notation	C1=CC(=CC=C1C2=C(C(=C(N2)C(F)(F)F)Br)C#N)Cl	PubChem 2023
Molecular weight	349.53 g/mol	PubChem 2023
Physical state	Solid	U.S. EPA 2013
Appearance	Off-white powder	U.S. EPA 2013
Melting point	252.3 - 253.4℃	U.S. EPA 2013
Boiling point	Not applicable	U.S. EPA 2013
Vapor pressure	3.45 x 10^{-10} mm Hg at 25℃	U.S. EPA 2013
Water solubility	Insoluble (0.16-0.17 mg/L)	U.S. EPA 2013
Dissociation constant	pKa = 7.08 at 26℃	U.S. EPA 2013
Density/specific gravity	1.714 g/cm^3 at 20℃	U.S. EPA 2013
Partition coefficient	Log K_{ow} = 3.5	U.S. EPA 2013

Toxicokinetics

Tralopyril is the N-dealkylated metabolite of chlorfenapyr (MRID 43492857), a pesticide for use on insects and mites. Tralopyril is the active form and functions as an uncoupler of oxidative phosphorylation in mitochondria, which disrupts ATP production and leads to cellular dysfunction and subsequent death of the organism (U.S. EPA 2014). No further information on toxicokinetics was identified in the public literature.

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

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

Tralopyril was assigned a score of Moderate for carcinogenicity based on weight of evidence from surrogate data and modeling. GreenScreen® criteria classify chemicals as a Moderate hazard for carcinogenicity when there is marginal evidence of carcinogenicity in animals (CPA 2018b). The confidence in the score is low as the data are not robust and some of the modeling results are of low reliability.

- 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 2014
    - The European Chemicals Agency (ECHA) stated in its opinion document on the application for approval of the active substance tralopyril, that tralopyril does not require classification for carcinogenicity, however the opinion appears to be based on insufficient data as opposed to negative data.
- U.S. EPA 2014
    - *Oral: Surrogate: Chlorfenapyr (CAS #122453-73-0):* Dogs (strain not specified) were exposed to chlorfenapyr in a chronic toxicity study per EPA Method 870.4100b. Animals were exposed at 0, 60, 120, or 240 ppm (equivalent to 0, 2.1,4.0, 8.7 mg/kg/day for males, and 0, 2.3, 4.5, or 10.1 mg/kg/day for females). The LOAEL was 8.7 mg/kg/day for males, and 10.1 mg/kg/day for females, based on decreased body weight. The NOAEL was 4.0 mg/kg/day for males, and 4.5 mg/kg/day for females (no further details were provided).
    - *Oral: Surrogate: Chlorfenapyr (CAS #122453-73-0):* Mice (strain not specified) were exposed to chlorfenapyr in a chronic toxicity study per EPA Method 870.4100b. Animals were exposed at 0, 20, 120, or 240 ppm (equivalent to 0, 2.8, 16.6 or 34.5 mg/kg/day for males, and 0, 3.7, 21.9, or 44.5 mg/kg/day for females). The LOAEL was 16.6 mg/kg/day for males, and 21.9 mg/kg/day for females, based on brain vacuolation and scabbing of the skin in males. There was no evidence of carcinogenicity. The NOAEL was 2.8 mg/kg/day for males, and 3.7 mg/kg/day for females (no further details were provided).
    - *Oral: Surrogate: Chlorfenapyr (CAS #122453-73-0):* Rats (strain not specified) were exposed to chlorfenapyr in a combined chronic toxicity / carcinogenicity study per EPA Method 870.4300. Animals were exposed at 0, 60, 300 or 600 ppm (equivalent to 0, 2.9, 15.0, or 30.8 mg/kg/day for males, and 0, 33.6, 18.6 or 37.0 mg/kg/day for females). High dose males had increased anemia, and females at 18.6 mg/kg/day and higher had decreased body weight. The NOAEL was 3.6 mg/kg/day based on decreased body weight in females. Authors concluded "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential" based on significant trends in liver tumors (adenomas and combined adenomas/carcinomas), malignant histiocytic sarcomas, and testicular cell tumors in male rats, and uterine polyps in female rats at the highest dose (no further details provided).
- Toxtree 2018
    - No experimental data were identified for this endpoint. Modeling was performed using Toxtree program v3.1.0. Tralopyril contains one structural alert for non-genotoxic carcinogenicity (Halogenated benzene) and no alerts for genotoxic carcinogenicity (Appendix D).
- VEGA 2023 (Appendix E)
    - If an external compound is beyond the defined scope of a given model, it is considered outside that model's applicability domain (AD) and cannot be associated with a reliable prediction (Sahigara 2007). Values for AD index (ADI) range from 0 (worst case) to 1 (best case). Generally, ADI values of > 0.70 indicate that the prediction has moderate or better predictivity (Gad 2016).
        - The VEGA CAESAR model predicts tralopyril to be non-carcinogenic. However, the reliability of this prediction is low based on a global ADI of 0.308. Therefore, the prediction is not included in the weight of evidence.
        - The VEGA ISS model predicts tralopyril to be carcinogenic. However, the reliability of this prediction is low based on a global ADI of 0.615. Therefore, the prediction is not included in the weight of evidence .

        - The VEGA IRFMN/Antares model predicts tralopyril to be non-carcinogenic. However, the reliability of this prediction is low based on a global ADI of 0.503. Therefore, the prediction is not included in the weight of evidence .
        - The VEGA IRFMN/ISSCAN-CGX model predicts tralopyril to be non-carcinogenic. However, the reliability of this prediction is low based on a global ADI of 0. Therefore, the prediction is not included in the weight of evidence.
        - The VEGA IRFMN oral classification model predicts tralopyril to be non-carcinogenic. However, the reliability of this prediction is low based on a global ADI of 0. Therefore, the prediction is not included in the weight of evidence.
        - The VEGA IRFMN inhalation classification model predicts tralopyril to be carcinogenic. The reliability of this prediction is high based on a global ADI of 0.862.
- U.S. EPA 2021
    - Tralopyril is predicted to have low to moderate carcinogenic concern when evaluated as a halogenated aromatic hydrocarbon by OncoLogic. The software was unable to evaluate the moiety of the five-carbon ring with bromine and fluorine substitutions (Appendix F).
- Based on the weight of evidence, a score of Moderate for carcinogenicity is assigned based on surrogate data and modeling. No data exist for tralopyril. Toxtree identified a structural alert for non-genotoxic carcinogenicity, and one of the VEGA models predicts tralopyril to be a carcinogen with high confidence (predictions from the remaining five VEGA models have low reliabilities). Surrogate data suggests some potential for carcinogenicity based on U.S. EPA conclusion of "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential" (U.S. EPA 2014).

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

Tralopyril was assigned a score of Low for mutagenicity/genotoxicity based on *in vitro* data for tralopyril, and *in vitro* and *in vivo* data for surrogate chlorfenapyr. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high based on the weight of evidence from multiple studies examining mutagenicity and clastogenicity.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2019
    - Negative results for mutagenicity were obtained in a mammalian cell mutation assay conducted in a manner similar to OECD Guideline 476. Chinese hamster ovary (CHO) cells were exposed to tralopyril (94%) in dimethyl sulfoxide up to cytotoxic and insolubility concentrations, with and without metabolic activation. No increase in the mutation frequency was observed in the presence or absence of metabolic activation.
    - Tralopyril was negative in a mouse micronucleus assay conducted in a manner similar to OECD Guideline 474 in male mice (5/group). Animals were exposed to tralopyril (>94% pure) in olive oil at doses of 0, 3, 6, or 12 mg/kg via gavage and bone marrow cells were harvested at 24 hours after exposure. Treatment did not induce an increase in the number of polychromatic or normochromatic erythrocytes containing micronuclei.
- U.S. EPA 2014
    - *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Chlorfenapyr tested negative in a bacterial reverse mutation assay per EPA Method 870.5100, using *Salmonella typhimurium* TA98,

TA100, TA1535, TA1537, and TA1538, and *Escherichia coli* WP_2 *uvr*A when exposed up to cytotoxicity (50 µg/plate, +/- S9) (no further details provided).

- *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Chlorfenapyr tested negative in an *in vitro* mammalian cell gene mutation assay per EPA Method 870.5300. Independently performed tests were negative up to a cytotoxic and precipitating concentration (500 µg/mL) with S9 activation, or the solubility limit (250 µg/mL) without S9 activation (no further details provided).
- *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Chlorfenapyr tested negative in an *in vitro* mammalian chromosome aberration test per EPA Method 870.5375. Results were negative up to 100 µg/mL without S9, or 25 µg/mL with S9; higher doses were cytotoxic with and without S9 (no further details provided).
- *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Chlorfenapyr tested negative in *in vivo* mammalian (mouse) micronucleus test per EPA Method 870.5395. Results were negative following a single gavage dose of 7.5 to 30 mg/kg in males, or 5-20 mg/kg in females. Clinical observations included deaths in males at the highest dose, and diarrhea in females at the highest dose. There was no evidence of cytotoxicity for the target organ (no further details provided).
- *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Chlorfenapyr tested negative in an *in vitro* test for unscheduled DNA synthesis per EPA Method 870.5550 using primary rat hepatocyte cultures exposed up to severely toxic concentrations (≥30 µg/mL) (no further details provided).

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

Tralopyril was assigned a score of Low for reproductive toxicity based on surrogate data. 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 low as it is reported study details were limited for the critical study.

- 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 2014
  - ECHA stated in its opinion document on the application for approval of the active substance tralopyril, that tralopyril does not require classification for reproductive toxicity. It is not clear however, if ECHA evaluated reproductive toxicity studies, or if the opinion is based on lack of effects on reproductive organs examined in standard repeated dose toxicity studies.
- U.S. EPA 2014
  - *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Rats (strain not specified) were exposed to chlorfenapyr in a two-generation reproductive toxicity study per EPA Test Guideline 870.3800 (GLP not specified). Rats were exposed at 0, 60, 300, or 600 ppm (equivalent to 0, 4.5, 22.2, and 44.0 mg/kg/day for males, and 0, 5.0, 24.5, and 48.3 mg/kg/day for females according to U.S. EPA). The parental LOAEL was 44.0 mg/kg/day in males, and 48.3 mg/kg/day in females, based on decreased body weights. The parental NOAEL was 22.2 mg/kg/day in males, and 24.5 mg/kg/day in females. The offspring LOAEL was 22.2 mg/kg/day in males, and 24.5 mg/kg/day in females, based on decreased pup weights. The offspring NOAEL was 4.5 mg/kg/day in males, and 5.0 mg/kg/day in females. Pup deaths were considered adverse at the highest dose in the F2 generation. The reproductive NOAEL was 44.0 mg/kg/day in males, and 48.3 mg/kg/day in females, the highest doses tested (no further details provided).

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *H*

Tralopyril was assigned a score of High for developmental toxicity based on effect in pups suspected to arise from exposure via lactation in a study conducted on the surrogate chlorfenapyr. GreenScreen® criteria classify chemicals as a High hazard for developmental toxicity when there are effects occurring via or on lactation (CPA 2018b). The confidence in the score is low study details are limited and EPA suggested the key study was "non-guideline pending positive control 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.
- U.S. EPA 2019
  - In a developmental toxicity study similar to OECD 414, time-mated Sprague-Dawley female rats (25/dose) were administered oral doses of tralopyril (94.6%) at 0, 5, 10, or 20 mg/kg/day via gavage on gestation days 6 to 19. Treatment related maternal toxicity at the highest dose included increased mortality, frequent salivation, reduced body weight gain, decreased uterine weight, decreased corrected body weight gain, increased resorptions (total, early and late) and decreased placental weight. Frequent salivation was also seen in animals at 10 mg/kg/day. Pups in the high and mid dose groups showed decreased mean fetal weight and exhibited disturbances and delayed ossification (non / incomplete / dumbbell / biparte) of various skeletal structures (skull, sternebrae), as well as supernumerary (14th) and wavy ribs. Authors considered delayed ossification and decreased fetal weight as secondary to maternal toxicity and identified the NOAEL for maternal and developmental toxicity at 5 mg/kg/day, and the LOAEL at 10 mg/kg/day based on significant clinical signs of toxicity and decreased pup weights, respectively.
- U.S. EPA 2014
  - *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Rats (strain not specified) were exposed to chlorfenapyr in a prenatal developmental toxicity study per EPA Method 870.3700a. Rats were exposed at 0, 25, 75, or 225 mg/kg/day. The NOAEL for maternal toxicity and developmental toxicity was 225 mg/kg/day, the highest dose tested (no further details provided).
  - *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Rabbits (strain not specified) were exposed to chlorfenapyr in a prenatal developmental toxicity study per EPA Method 870.3700b. Animals were exposed at 0, 5, 15, or 30 mg/kg/day. The NOAEL for maternal toxicity and developmental toxicity was 30 mg/kg/day, the highest dose tested (no further details provided).
  - *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Rats (strain not specified) were exposed to chlorfenapyr in a developmental neurotoxicity study conducted in a manner similar to EPA Method 870.6300, but non-guideline pending positive control data. Rats were exposed at 0, 5, 10, or 15 mg/kg/day. The NOAEL for maternal toxicity was 15 mg/kg/day, the highest dose tested. The LOAEL for developmental effects was 10 mg/kg/day based on increased pup deaths and decreased motor activity. At the high dose, vacuolation of the white matter and decreased hippocampus size were observed in several areas of the brain at postnatal day (PND) 22. The developmental NOAEL was 5 mg/kg/day (no further details provided).
- Based on the weight of evidence, a score of High for developmental toxicity is assigned based on suspected effects via lactation in a study conducted on the surrogate chlorfenapyr. One study on tralopyril demonstrated onset of developmental effects at the same dose that caused maternal toxicity, and two studies on surrogate chlorfenapyr did not find developmental toxicity. However, a third study on surrogate chlorfenapyr demonstrated increased pup deaths, and decreased motor activity, at a lower dose than the onset of maternal toxicity, and EPA attributed the effects as

suspected to be the result of postnatal exposure through lactation (U.S. EPA 2014). Further, tralopyril is expected to be more toxic to maternal animals and offspring (JMPR 2013).

Endocrine Activity (E) Score (H, M, or L): DG
Tralopyril was assigned a score of Data Gap for endocrine activity based on lack of studies specifically targeting the endocrine system *in vivo*.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2023b
  - Tralopyril was active in 1/4 estrogen receptor (ER) assays, 2/3 androgen receptor (AR) assays, 7/24 steroidogenesis assays, and 1/7 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century (Appendix G).
- DTU 2023
  - Modeling in the Danish QSAR database provides the following results that are within the applicability domains of the models (Appendix H):
    - Tralopyril is predicted to be negative for estrogen receptor α binding (full training set, human *in vitro*), estrogen receptor α activation (human *in* vitro), and estrogen receptor activation, CERAPP data (*in vitro*), but positive for estrogen receptor α binding (balanced training set, human *in vitro*) in LeadScope. Also predicted to be negative for estrogen receptor α activation (human *in* vitro) in Battery and SciQSAR and negative for estrogen receptor α binding (balanced training set, human *in vitro*) in SciQSAR.
    - Tralopyril is predicted to be negative for androgen receptor inhibition (human *in vitro*) by the model battery consisting of negative and in domain predictions by the Battery, Leadscope, and SciQSAR models. Also predicted to be negative for androgen receptor activation, CoMPARA data (*in vitro*) by Leadscope.
    - Tralopyril is predicted to be negative for thyroperoxidase (TPO) inhibition (QSAR1 and QSAR2, rat *in vitro*) by the Leadscope models.
- VEGA 2023 (Appendix I)
  - The VEGA estrogen receptor relative binding affinity (IRFMN) model predicts tralopyril to be active. However, the reliability of this prediction is low based on a global ADI of 0. Therefore, the prediction is not reliable.
  - The VEGA estrogen receptor-mediated effect (IRFMN/CERAPP) model predicts tralopyril to be non-active. The reliability of this prediction is high based on a global ADI of 0.875.
  - The VEGA androgen receptor-mediated effect (IRFMN/COMPARA) model predicts tralopyril to be non-active. The reliability of this prediction is high based on a global ADI of 0.909.

<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
Tralopyril was assigned a score of very High for acute toxicity based on oral LD_{50} values of 27 – 29 mg/kg in rats, inhalation LC_{50} values of 0.5 mg/L and less in rats, severe acute oral toxicity designation

[GHS Category 1] (U.S. EPA 2019), H300 (ECHA 2014), and H330 (Fatal if swallowed) (ECHA 2014). GreenScreen® criteria classify chemicals as a very High hazard for acute toxicity when data meet GHS Category 1 or 2 classification, which includes H300 and H330 (CPA 2018b). The confidence in the score is high based on reliable data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS-Korea: Acute toxicity (oral) Category 2, H300 – Fatal if swallowed.
    - GHS-New Zealand: 6.1B (oral) – acutely toxic.
    - GHS-Korea: Acute toxicity (inhalation) Category 3, H331 – Toxic if inhaled.
- JMPR 2013
  - *Oral:* LD_{50} = 27 mg/kg and 29 mg/kg in male and female rats, respectively.
    - According to GHS (UN 2019), oral LD_{50} values of 5 – 50 mg/kg warrant classification to GHS Category 2.
- U.S. EPA 2019
  - *Oral:* No acute oral toxicity value was provided but U.S. EPA stated that tralopyril has been shown to have severe acute toxicity by the oral route and is classified to Toxicity Category 1.
  - *Dermal:* LD_{50} > 2,000 mg/kg (rabbits) (EPA Method 870.1200), meets EPA category III (low toxicity).
  - *Inhalation:* $LC_{50} \leq 510$ mg/m^3 (rats) equivalent to 0.5 mg/L (EPA Method 870.1300), meets EPA category II (moderate toxicity).
    - According to GHS (UN 2021), inhalation LC_{50} values of 0.5 mg/L or less warrant classification to GHS Category 1 or 2.
- ECHA 2014
  - The proposed classifications for tralopyril according to CLP Regulation, by ECHA in its opinion document on the application for approval of the active substance tralopyril, included Acute Tox. 2 with hazard statement of H300 (Fatal if swallowed), Acute Tox. 3 with hazard statement of H311 (Toxic in contact with skin) and Acute Tox. 2 with hazard statement of H330 (Fatal if inhaled).

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

Tralopyril was assigned a score of Data Gap for systemic toxicity (single dose) based on lack of data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- No data identified.

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

Tralopyril was assigned a score of High for systemic toxicity (repeated dose) based on an inhalation LOAEC of 0.02 mg/L generated from a 90-day study in rats. GreenScreen® criteria classify chemicals as a High hazard for systemic toxicity (repeated dose) when the oral LOAEL is ≤ 10 mg/kg/day, dermal LOAEL is ≤ 20 mg/kg/day, inhalation LOAEC is ≤ 0.02 mg/L for dust or when they are classified to GHS Category 1 (CPA 2018b). The confidence in the score is high 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.
- U.S. EPA 2019
  - *Oral:* In a previously described developmental toxicity study similar to OECD 414, time-mated Sprague-Dawley female rats (25/dose) were administered oral doses of tralopyril (94.6%) at 0, 5, 10, or 20 mg/kg/day via gavage on gestation days 6 to 19. Treatment related maternal toxicity effects were seen in dams exposed to 20 mg/kg/day. These included increased mortality, frequent salivation, reduced body weight gain, decreased uterine weight, decreased corrected body weight gain, increased resorptions (total, early and late) and decreased placental weight. Frequented salivation was also seen in animal at 10 mg/kg/day dose group. Based on this, a NOAEL of 5 mg/kg/day for maternal toxicity was established.
  - *Oral:* In a 90-day repeated dose toxicity study conducted in a manner similar to OPPTS 870.3100, Sprague-Dawley rats (10/sex/dose group) were fed diets containing tralopyril (94.6%) at concentrations of 0, 80, 250, or 750 ppm. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, in males and to 6.3, 20.9, or 62.0 mg/kg/day, in females as calculated by the authors. An additional study was performed with 10 Sprague-Dawley rats/sex/dose at the same concentration as above with 5 rats/sex/dose undergoing terminal sacrifice by *in situ* perfusion after anesthesia after 3 months of exposure and 5 rats/sex/dose undergoing a 4-week recovery period. The animals were evaluated for clinical signs of toxicity, body weight, food consumption, hematology, clinical chemistry, gross pathology, and histopathology. No mortalities or clinical signs of toxicity were observed but several treatment-related effects were noted. A decrease in food consumption was seen in both males and females administered 750 ppm in the first week of treatment. The body weight gains were low throughout the study, likely due to low food consumption. Hematology analysis revealed only minor (<10% difference from controls) effects in high-dose animals, except for a 15% increase in platelets in the males with a corresponding increase in females that was not significant (9%). All hematological values were comparable to controls after the 4-week recovery period. There were statistically significant increases in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in males at the mid- and high-dose groups, which correlated to increased relative liver weights in the main group. Females displayed a lesser increase in ALP with only the high-dose group demonstrating a statistically significant change. A statistically significant increase in ALP was also seen in males at 80 ppm. The urinary excretion of the phosphorus, urea, and magnesium and serum total cholesterol were also statistically significantly increased in both males and females at high dose. Animals in this dose group have shown a statistically significant decrease in serum glucose. Recovery groups presented no significant clinical chemistry parameter abnormalities, indicating full recovery from all treatment related effects. Differences in creatinine and triglycerides were generally <10% compared to the main control group and were thus attributed to biological variation. Mean terminal body weight was statistically significantly decreased in mid- and high-dose males and in high-dose females, which affected the absolute and/or relative weights of several organs. These changes in organ weights included increases in relative brain weights (mid- and high-dose males; high-dose females) and liver weights (mid- and high-dose males; all treated females). However, the changes in ALP and ALT enzyme were reversible after 4 weeks and the increases in the liver were likely due to increased metabolism of the test article. Treatment-related microscopic findings in the main group included minimal to slight mucosal hyperplasia of the duodenum in the mid- and high-dose males and females that were reversible after 4 weeks. Neurotoxicity was also observed, which was described in the neurotoxicity section below. Based on reduced body weight and body weight gain, reduced food consumption,

hematology, clinical chemistry, and organ weights, the study authors identified a LOAEL of 16.2 mg/kg/day and a NOAEL of 5.2 mg/kg/day in male rats. A LOAEL of 6.3 mg/kg/day, the lowest dose tested, was established for female rats based on microscopic findings of the brain and spinal cord. ToxServices established the systemic effect LOAEL at 62.0 mg/kg/day and the NOAEL at 20.9 mg/kg/day for females based on reduced body weight, clinical chemistry, and urinalysis. Neurotoxic effects are discussed in the neurotoxicity section below.

- *Dermal:* In a 90-day repeated dose toxicity study conducted similar to OECD Guideline 414, tralopyril (94.6%) was applied dermally to the shaved skin of male and female rats (10/sex/dose) at doses of 0, 100, 300, or 1,000 mg/kg/day, 6 hours/day for 5 days/week. No toxicologically relevant changes were reported. A dose dependent dermal irritation was observed in both males and females at the mid- and high-dose group. Consequently, the study authors identified a dermal NOAEL of 100 mg/kg/day for local effects, and a NOAEL of >1,000 mg/kg/day for systemic toxicity, the highest dose tested.
- *Inhalation:* In a two-phase, 90-day inhalation toxicity and neurotoxicity study in rats conducted according to the EPA OPPTS 870.3465, 870.6200 Guidelines, Sprague-Dawley rats (10/sex/dose in Phase I; 12/sex/dose in Phase II) were exposed (nose only) to tralopyril (94% pure) at air concentrations of 0, 20, 40, and 80 mg/m^3 for 6 hours/day, 7 days/week. The particle size was within the accepted range. Neurotoxicity effects are discussed in the neurotoxicity (repeat dose) section below. In the phase I study, signs of toxicity were seen in both males and females at all doses and these included brown staining on the neck, forelimb, abdominal area thoracic area, inguinal area, hindlimb, rump, anogenital area, urogenital area, and tail. Mean body weight was reduced in males at all doses and in females at mid- and high- dose groups. Tralopyril treatment caused effects on the nasal passages including inflammation, ulcerations, and exudate in males, and inflammation, epithelial hyperplasia, hyperkeratosis, and degeneration of the olfactory epithelium in females at 20 mg/m^3 (the lowest concentration tested). Based reduced body weights and pathological changes in the respiratory tract, a LOAEC of 20 mg/m^3 was established for male and female rats.

- Based on the above data, the inhalation LOAEC identified for tralopyril was 20 mg/m^3/6h/day in a 90-day study, which is equivalent to 0.02 mg/L/6h/day. The concentration is equal to the GHS category 1 cut-off value of 0.02 mg/L/6h/day for dust. For the oral route, data straddle the GHS Category 1 and 2 guidance values based on a LOAEL of 16.2 mg/kg/day (males) and a NOAEL of 5.2 mg/kg/day. Therefore, based on the weight of evidence, a score of High for systemic toxicity is assigned based on a GHS Category 1 systemic toxicity classification for tralopyril via the inhalation route of exposure.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *vH*
Tralopyril was assigned a score of Very High for neurotoxicity (single dose) based on surrogate data which provide a LOAEL of 45 mg/kg/day for decreased motor activity on the day of dosing. This meets GHS category 1 classification (LOAEL ≤ 300 mg/kg/day). GreenScreen® criteria classify chemicals as a Very High hazard for neurotoxicity (single dose) when sufficient data are available and meet the criteria for GHS Category 1 classification (CPA 2018b). The confidence in the score is low as limited study details were provided.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2014

- *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Rats (strain not specified) were exposed to chlorfenapyr in an acute neurotoxicity screening battery per EPA Method 870.6200a. Animals were exposed at 0, 45, 90 or 180 mg/kg/day. The LOAEL was 45 mg/kg/day based on decreased motor activity on day of dosing at the lowest dose. The NOAEL was not established (no further details provided).

- Based on the available data, a score of Very High was assigned for neurotoxicity (single dose). Limited experimental data were identified for the surrogate chlorfenapyr, indicating significant effects on motor activity in rats at doses as low as 45 mg/kg/day. As the anticipated toxicity of tralopyril is anywhere from 7 to 45 times greater than chlorfenapyr (JMPR 2013), ToxServices conservatively assigned a score of Very High.

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

Tralopyril was assigned a score of High for neurotoxicity (repeated dose) based on data meeting GHS Category 1 classification criteria for neurotoxicity by the oral route of exposure. GreenScreen® criteria classify chemicals as a High hazard for neurotoxicity (repeated dose) when there are adequate data and they meet the criteria for GHS Category 1 classification (CPA 2018b). The confidence in the score is high 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.
- U.S. EPA 2019
  - *Oral:* In the previously described 90-day repeated dose toxicity study conducted in a manner similar to OPPTS 870.3100, Sprague-Dawley rats (10/sex/dose group) were fed diet containing tralopyril (94.6%) at concentrations of 0, 80, 250, or 750 ppm. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, in males and to 6.3, 20.9, or 62.0 mg/kg/day, in females as calculated by the authors. Changes in organ weights included increases in relative brain weights (mid- and high-dose males; high-dose females). The relative brain weights in the high-dose animals remained elevated after the recovery period, though the values were only statistically significant in males. However, frank effects were noted in the brain at necropsy in these recovery group animals. Microscopic findings of the brain and spinal cord were observed in the main group as follows: minimal to moderate multifocal vacuolation of the brain in the mid- and high-dose males and females as well as the low-dose females. These were spongiform myelinopathy in the central and the proximal peripheral nervous system. The most severe lesions were multifocal vacuolation of the white matter and intramyelinic vacuolation of the peripheral nerves in the cauda equina (lumbar cord and root fibers). No treatment related changes in behavior or parasympathetic movements (e.g. tremors, convulsions, impaired gait, posture, response to handling, activity, or arousal) were seen. After 4 weeks of no treatment, microscopic lesions were still observed in the brain and lumbar region. Minimal multifocal vacuolization of the brain in high-dose males and minimal to slight multifocal vacuolization of the brain in mid- and high-dose females were observed; minimal to slight multifocal vacuolization of the lumbar in mid- and high-dose males and minimal to moderate multifocal vacuolization of the lumbar in all treated females. The lesion severity increased with dose. Based on the microscopic findings of the brain and spinal cord, the study authors identified a LOAEL of 6.3 mg/kg/day for female rats, the lowest dose tested.
  - *Inhalation:* In a two-phase, 90-day inhalation toxicity and neurotoxicity study in rats conducted according to the EPA OPPTS 870.3465, 870.6200 Guidelines, Sprague-Dawley rats (10/sex/dose in Phase I; 12/sex/dose in Phase II) were exposed (nose only) to tralopyril

(94% pure) at air concentrations of 0, 20, 40, and 80 mg/m^3 for 6 hours/day, 7 days/week. The particle size was within the accepted range. In the phase II study, animals underwent a full neurobehavioral examination (functional observational battery [FOB] and motor activity testing) the day before dosing, and at weeks 3, 7, and 12. Cholinesterase activity was not determined. At study termination, 6 animals/sex/dose in the controls and 80-mg/m^3 groups were perfused *in situ* for neuropathological examination of the central and peripheral nervous system tissues. An increase in mortality among animals receiving the 80 mg/m^3 was seen. Decreased motor activity was observed in males at 40 and 80 mg/m^3 and axonal degeneration in the peroneal nerve was observed in both males and females at 80 mg/m^3. Based on decreased motor activity, the neurotoxicity NOAEL of 20 mg/m^3 and LOAEC of 40 mg/m^3 were established for male rats. In females, the neurotoxicity NOAEC of 40 mg/m^3 and LOAEC of 80 mg/m^3 were established based on axonal degeneration observed in the peroneal nerve.

- Based on the above data, neurotoxicity was observed in animals exposed to tralopyril via oral and inhalation routes of exposure. The lowest inhalation neurotoxicity LOAEC identified for tralopyril was 40 mg/m^3/6h/day for male rats in a 90-day study, which is equivalent to 0.04 mg/L/6h/day, and within the guidance values for GHS Category 2 for dust (0.02-0.2 mg/L/6h/day). However, the oral neurotoxicity LOAEL (6.3 mg/kg/day) meets the GHS Category 1 guidance value (≤ 10 mg/kg/day). Therefore, tralopyril is classified to GHS Category 1 for neurotoxicity via the oral route of exposure.

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

Tralopyril was assigned a score of Low for skin sensitization based on lack of skin sensitization in exposed guinea pigs. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data exist and meet the criteria for GHS not classified (CPA 2018b). The confidence in the score is low as no information was available regarding test substance concentration in guinea pigs and due to mixed results across multiple skin sensitization models.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2019
  - Tralopyril did not cause skin sensitizing in guinea pigs when tested according to EPA OPPTS 870.2600 Guideline (no further details were provided).
- U.S. EPA 2014
  - *Surrogate: Chlorfenapyr (CAS #122453-73-0):* Not sensitizing to guinea pigs when tested per EPA method 870.2600.
- Payne and Walsh 1994
  - Tralopyril possesses no known structural alerts for skin sensitization (Appendix J).
- LabMol 2020
  - Only one prediction was within the applicability domain (AD) (confiability 62%) and predicted tralopyril to be sensitizing in a human Cell Line Activation Test (h-CLAT) cellular response *in vitro* assay (Appendix K).
- Toxtree 2018
  - Tralopyril contains no structural alerts for skin sensitization (Appendix L).
- VEGA 2023 (Appendix M)
  - The VEGA CAESAR model predicts tralopyril to be sensitizing. However, the reliability of this prediction is low based on a global ADI of 0.241. Therefore, the prediction is not included in the weight of evidence.

    - The VEGA IRFMN/JCR model predicts tralopyril to be non-sensitizing. However, the reliability of this prediction is low based on a global ADI of 0. Therefore, the prediction is not included in the weight of evidence.
- OECD 2023
    - Positive prediction for protein binding alert for skin sensitization – SNAr (Nucleophilic aromatic substitution on activated halogen, cyano, isocyano, sulfo, sulfonyl groups, etc. Halogenated five membered aromatic compounds) (Appendix N).
- DTU 2023
    - Modeling in the Danish QSAR database provides the following results that are within the applicability domains of the models (Appendix O):
        - Tralopyril is predicted to be negative for skin sensitisation GHS/CLP at least Cat. 1, LLNA-based (open data and REACH-registrations) and negative for skin sensitisation GHS/CLP Cat. 1A, LLNA-based (open data and REACH-registrations) in Leadscope.
- Based on the weight of evidence, a score of Low is assigned based on negative results for skin sensitization in guinea pigs. Tralopyril has no structural alerts for skin sensitization as identified by Payne and Walsh (1994) and Toxtree (2018). Positive modeling predictions for h-CLAT cellular response in LabMol (2020) indicates a potential for skin sensitization. OECD Toolbox (2023) also identifies a structural alert for protein binding to skin sensitization based on nucleophilic aromatic substitution in the halogenated five membered aromatic ring of tralopyril. Two negative predictions for skin sensitization were identified within the applicability domain of the Danish QSAR (DTU 2023) database output. In general, the negative experimental data take precedence over the positive modeled results. Therefore, the concern for skin sensitization is low.

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

Tralopyril was assigned a score of Low for respiratory sensitization based on extrapolation from dermal sensitization data and modeling. GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when adequate data exist and meet the criteria for GHS not classified (CPA 2018b). The confidence in the score is low as this evaluation does not include non-immunologic mechanisms of respiratory sensitization, and no specific data are available for respiratory sensitization.

- 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
    - Tralopyril does not contain any structural alerts for respiratory sensitization (Appendix N).
- DTU 2023
    - Tralopyril was outside of the applicability domain for respiratory sensitization prediction (Appendix O).
- Based on the weight of evidence and guidance from ECHA regarding assessment of respiratory sensitization potential, a score of Low was assigned. The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). As tralopyril was not sensitizing to the skin (see skin sensitization section above), and a literature search did not find any human evidence of respiratory sensitization by tralopyril, and as tralopyril does not contain any structural

alerts for respiratory sensitization (OECD 2023), tralopyril is not expected to be a respiratory sensitizer.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): *M*
Tralopyril was assigned a score of Moderate for skin irritation/corrosivity based on EPA classification as a mild skin irritant. GreenScreen® criteria classify chemicals as a Moderate hazard for skin irritation/corrosivity when they are mild irritants (CPA 2018b). The confidence in the score is low as data are insufficiently detailed to discern if the data would translate to GHS Category 3 or not classified. As a conservative measure, ToxServices assigned GHS Category 3, mild irritant.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2019
  - In an acute dermal toxicity study conducted according to EPA OPPTS 870.2500 Guideline, tralopyril was mildly irritating to the skin of rabbits based on very slight erythema, but no edema at 72 hours (no further details provided). EPA designated tralopyril as a mild skin irritant (Toxicity Category IV).

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): *M*
Tralopyril was assigned a score of Moderate for eye irritation/corrosivity based on EPA classification as a mild eye irritant. GreenScreen® criteria classify chemicals as a Moderate hazard for eye irritation/corrosivity when they are mild irritants (CPA 2018b). The confidence in the score is low as data are insufficiently detailed to discern if the data would translate to GHS category 2B or not classified. As a conservative measure, ToxServices assigned GHS Category 2B, mild irritant.
Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2019
  - Tralopyril was mildly irritating to rabbit eyes in an ocular irritation study conducted according to EPA OPPTS 870.2400 Guideline (no further details provided). EPA designated tralopyril as a mild eye irritant (Toxicity Category III).

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): vH
Tralopyril was assigned a score of Very High for acute aquatic toxicity based on LC/EC_{50} values < 1 ppm (1 mg/L) in fish, daphnia and algae, and H400 (Very toxic to aquatic life) hazard classification recommended by numerous authoritative bodies and notifiers within the ECHA C&L Inventory (ECHA 2020). GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when LC/EC_{50} values are < 1 ppm in any one species, and/or they meet the criteria for hazard classification H400 (CPA 2018b). The confidence in the score is high based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS-Japan – Hazardous to the aquatic environment (acute) – Category 1 (H400)
    - GHS-Korea - Hazardous to the aquatic environment (acute) – Category 1 (H400 – Very toxic to aquatic life)
    - GHS-New Zealand – 9.1A (fish) – Very ecotoxic in the aquatic environment

- U.S. EPA 2019
  - 96 hr LC_{50} = 1.3 ppb in Rainbow trout (*Oncorhynchus mykiss)* (94.6% purity)
  - 96 hr LC_{50} = 3.2 ppb in Bluegill sunfish (*Lepomis macrochirus)* (94.6% purity)
  - 96 hr LC_{50} = 1.4 ppb in Zebra fish *(Danio rerio)* (98.1% purity)
  - 96 hr LC_{50} = 23.7 ppb in Sheepshead minnow (*Cyprinodon variegatus*) (94.6% purity)
  - 96 hr LC_{50} > 950 ppb in Sheepshead minnow (*C. variegatus*) (93% purity)
  - 96 hr LC_{50} > 89,000 ppb in Sheepshead minnow (*C. variegatus*) (94.5% purity)
  - 96 hr LC_{50} > 16,000 ppb in Sheepshead minnow (*C. variegatus*) (96% purity)
  - 48 hr EC_{50} = 1.5 ppb in *Daphnia magna* (94.6% purity)
  - 48 hr EC_{50} = 1,630 ppb in *D. magna* (93% purity)
  - 48 hr EC_{50} < 600 ppb in *D. magna* (93% purity)
  - 48 hr EC_{50} = 16,800 ppb in *D. magna* (98% purity)
  - 48 hr EC_{50} = 3,510 ppb in *D. magna* (97% purity)
  - 96 hr EC_{50} = 11 ppb in green algae (*Pseudokirchneriella subcapitata*) (94.6% purity)
  - 96 hr EC_{50} = 4.49 ppb in green algae (*R. subcapitata*) (94.6% purity)
  - 96 hr EC_{50} = > 4,620ppb in green algae (*R. subcapitata*) (93% purity)
  - 96 hr EC_{50} = > 1,990 ppb in green algae (*Ra. subcapitata*) (98% purity)
- ECHA 2014
  - The proposed classifications for tralopyril according to CLP Regulation by ECHA in its opinion document on the application for approval of the active substance tralopyril, included Acute Aquatic 1 with a hazard statement of H400.

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): vH
Tralopyril was assigned a score of very High for chronic aquatic toxicity based on LC/EC_{50} values < 0.1 ppm (0.1 mg/L) in fish and daphnia (ECHA 2020). GreenScreen® criteria classify chemicals as a very High hazard for acute aquatic toxicity when LC/EC_{50} values are < 1 ppm in any one species (CPA 2018b). The confidence in the score is high based on experimental data for two trophic levels sufficient to assign the worst score.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2019
  - LOEC and NOAEC in *D. rerio* of 0.37 and 0.17 ppb, respectively (fish, 33d)
  - *Tralopyril technical grade:* LOAEC and NOAEC in *D. magna* of 0.57 and 0.20 ppb, respectively (invertebrate, 21d)
  - *Surrogate: CL 322,250:* LOEC and NOAEC in *D. rerio* of 140 and 69 ppb, respectively (fish, 35d)
  - *Surrogate: CL 322,250:* LOAEC and NOAEC in *D. magna* of 540 and 300 ppb, respectively (invertebrate, 21d)

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *vH*
Tralopyril was assigned a score of Very High for persistence based on limited data identifying degradants, and modeling which suggests partitioning of tralopyril and its degradants to soil and sediment with half lives in the range of 180-360 days (U.S. EPA 2019). GreenScreen® criteria classify chemicals as a Very High hazard for persistence when the dominant compartment is soil/sediment and half-lives exceed 180 days (CPA 2018b). The confidence in the score is reduced as it is based on modeling.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2019
  - Once released into fresh or salt water, tralopyril is expected to form the primary, major and persistent degradant CL322,250 (the defluorinated parent compound). CL322,250 is expected to undergo some degradation to compounds with lower molecular weight, higher water solubility, and lower log K_{ow}, but neither the parent compound nor degradants are expected to volatilize from water.
  - Hydrolysis half-lives for tralopyril ranged from 14-175 days at pH 5 to 0.1-0.6 days at pH 9 in seawater; but CL322,250 did not degrade by hydrolysis.
  - Photodegradation for tralopyril was 1 day in distilled water and humic acid water, 0.025 days (36 minutes) for CL322,250.
  - Metabolic half-lives for tralopyril under anaerobic conditions were 29 and 0.7 days in freshwater and saltwater, and 12 and 0.6 days under aerobic conditions. Half-lives for CL322,250 were 23-31 days in these conditions.
  - Another major degradant is CL322,248 in anerobic conditions.
- Danish EPA 2023
  - Half-lives for CL322,250 were predicted to be 30-87 days in water/sediment systems.
- U.S. EPA 2017
  - The BIOWIN model predicts that tralopyril is not readily biodegradable. The level III fugacity model predicts partitioning to soil at 83.9% and sediment at 12.9%, with half lives of 360 days and 162 days, respectively (Appendix P).

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL
Tralopyril was assigned a score of Very Low for bioaccumulation based on a measured BCF and log K_{ow}. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the BCF is ≤ 100 and log K_{ow} is ≤ 4 (CPA 2018b). Although the bioconcentration study is not well detailed, the results are consistent with modeled values using the experimental log K_{ow}. The confidence in the score is high based on an experimental log K_{ow}.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2013
  - Tralopyril has a measured log K_{ow} value of 3.5 according to EPA OPPTS 830.7550 Guideline. A bioconcentration factor (BCF) of 3.2-32 in fish was also measured for tralopyril in carp at acidic conditions, which are below the pKa of 7.08, therefore tralopyril would not have ionized and would have had maximum bioconcentration.
- U.S. EPA 2017
  - The predicted BCF was 94.69 L/kg wet-wt based on a log K_{ow} of 3.50 using the regression-based method, and 301.1 for the upper trophic level using the Arnot-Gobas model considering biotransformation (Appendix P).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): L
Tralopyril was assigned a score of Low for reactivity based on experimental data and no structural alerts for reactivity. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when adequate

data exist and meet the criteria for GHS not classified (CPA 2018b). The confidence in the score was high based on 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.
- U.S. EPA 2013
  - Tralopyril is expected to be stable under normal conditions of use and not reactive when tested according to EPA OPPTS 830.6313 Guideline.
- No other measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of tralopyril. These procedures are listed in the GHS (UN 2021).
  - Based on the structure of its components or moieties, tralopyril is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix Q).
  - Based on the structure of its components or moieties, tralopyril is not considered to have oxidizing properties as it does not contain any structural groups known to be correlated with a tendency to react exothermally with combustible materials. Specifically, organic substances which contain oxygen, fluorine, or chlorine where these elements are chemically bonded only to carbon or hydrogen, classification as an oxidizing liquid need not be applied as tralopyril is a solid.

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

Tralopyril was assigned a score of Low for flammability based on experimental data. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data exist and meet the criteria for GHS not classified (CPA 2018b). The confidence in the score was high based on 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.
- U.S. EPA 2013
  - Tralopyril was not combustible/flammable when tested according to EPA OPPTS 830.6315 Guideline.

Use of New Approach Methodologies (NAMs)[11] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for carcinogenicity, endocrine activity, skin and respiratory sensitization, persistence, and bioaccumulation, and *in vitro* testing for genotoxicity and endocrine activity. 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 2020, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

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

As shown in Table 5, Type I (input data) uncertainties in tralopyril's NAMs dataset include limited, or lack of, experimental data for carcinogenicity, endocrine activity, skin sensitization, respiratory sensitization, and persistence, and lack of established test methods for respiratory sensitization. Tralopyril's Type II (extrapolation output) uncertainties include lack of defined applicability domains of some modeling software examining structural alerts, inability of OncoLogic to evaluate the entire structure of the target compound, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions and *in vitro* receptor binding activity assays, and the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization. Some of tralopyril'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	**Carcinogenicity**: Only limited experimental data are available. **Endocrine activity:** No *in vivo* data for hormone signaling pathways are available. **Skin sensitization:** Available experimental data have very limited reported details. **Respiratory sensitization**: No experimental data are available and there are no validated test methods. **Persistence:** No experimental data are available on environmental partitioning and half-lives of ultimate degradation in each compartment.

[11] 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).

Type II Uncertainty: Extrapolation Output	**Carcinogenicity**: Toxtree only identifies structural alerts (SAs), and no applicability domain can be defined (Toxtree 2018). OncoLogic is only able to evaluate a partial structure of the compound. **Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[12]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[13] The mammalian cell gene mutation assay (as defined in OECD Guideline 490) cannot reliably detect aneugens, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells)[14]. 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[15]. The *in vitro* UDS assay detects "longpatch repair" but is less sensitive for detection of "shortpatch repair". Mutagenic events may result from non-repair, misrepair or misreplication of DNA lesions, and UDS gives no indication of fidelity of the repair process. It is possible that a mutagen interacts with DNA but damage is not repaired by an excision repair process.[16] **Endocrine activity:** The *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* modeling of receptor binding is unknown due to lack of consideration of metabolism and other toxicokinetic factors. EDSP Tox 21 assays do not cover all critical endocrine pathways. **Skin sensitization:** The Payne and Walsh structural alerts and OECD Toolbox structural alerts don't define applicability domains. **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.

[12] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[13] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[14] https://www.oecd-ilibrary.org/docserver/9789264264908-en.pdf?expires=1622037214&id=id&accname=guest&checksum=F0669770FC98B49A32E3AFBA1A4D86F5
[15] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352
[16] https://www.oecd-ilibrary.org/environment/test-no-486-unscheduled-dna-synthesis-uds-test-with-mammalian-liver-cells-in-vivo_9789264071520-en#:~:text=The%20purpose%20of%20the%20unscheduled,physical%20agents%20in%20the%20liver.

Endpoint	NAMs Data Available and Evaluated? (Y/N)	Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)
Carcinogenicity	Y	*In silico* modeling: VEGA/Toxtree/OncoLogic™
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays *In silico* modeling: Danish QSAR/VEGA
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	Y	*In silico* modeling: VEGA/Payne and Walsh (1994) structural alerts/LabMol/Danish QSAR/Toxtree/OECD Toolbox
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts/Danish QSAR
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	Y	*In silico* modeling: EPI Suite™
Bioaccumulation	Y	*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 Tralopyril (CAS #122454-29-9)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/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	Tralopyril	122454-29-9	*M*	L	*L*	*H*	DG	vH		H	*vH*	H	*L*	*L*	*M*	*M*	vH	vH	*vH*	vL	L	L

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Tralopyril	1

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

Table 6

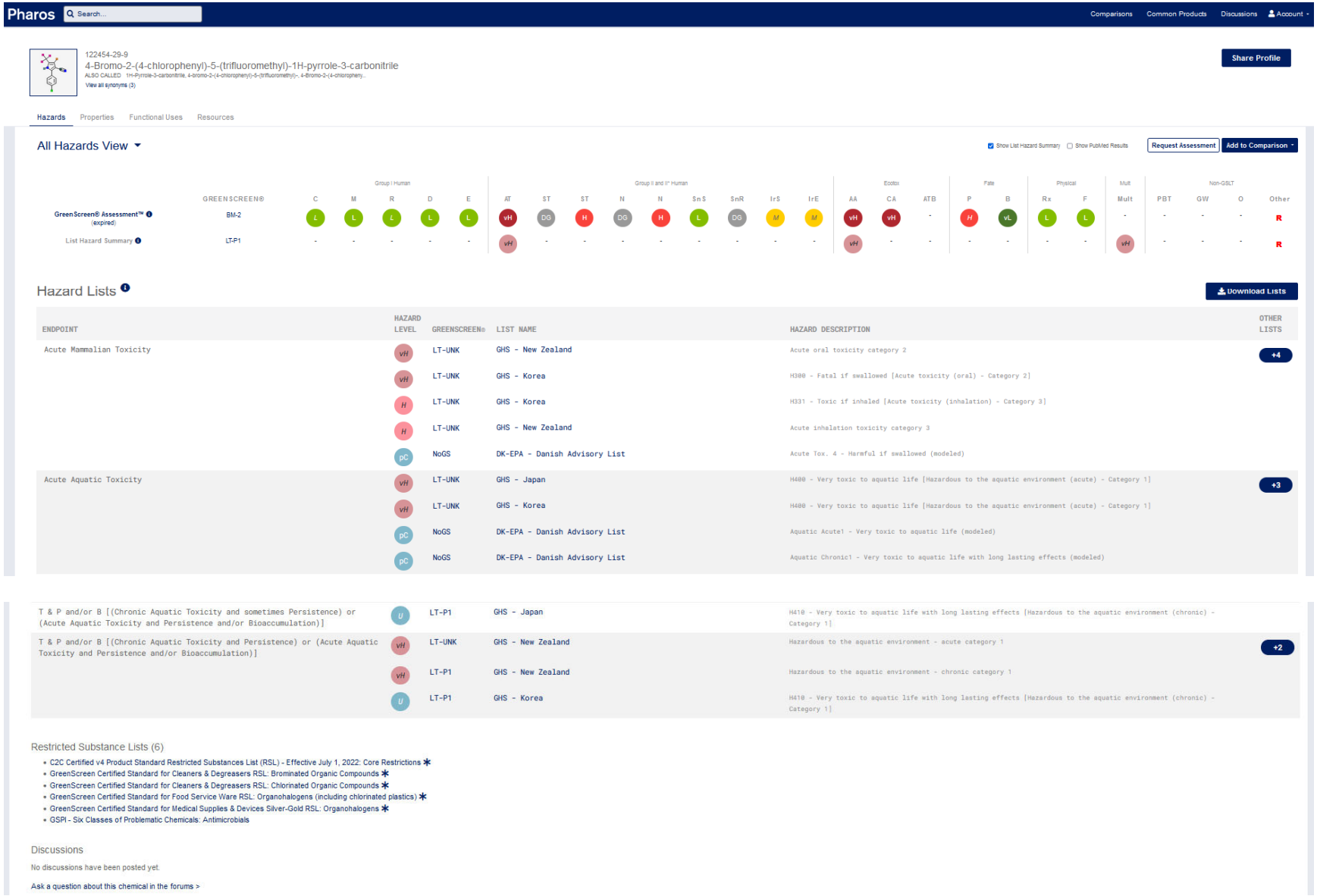
Chemical Name	Final GreenScreen® Benchmark Score
Tralopyril	1

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

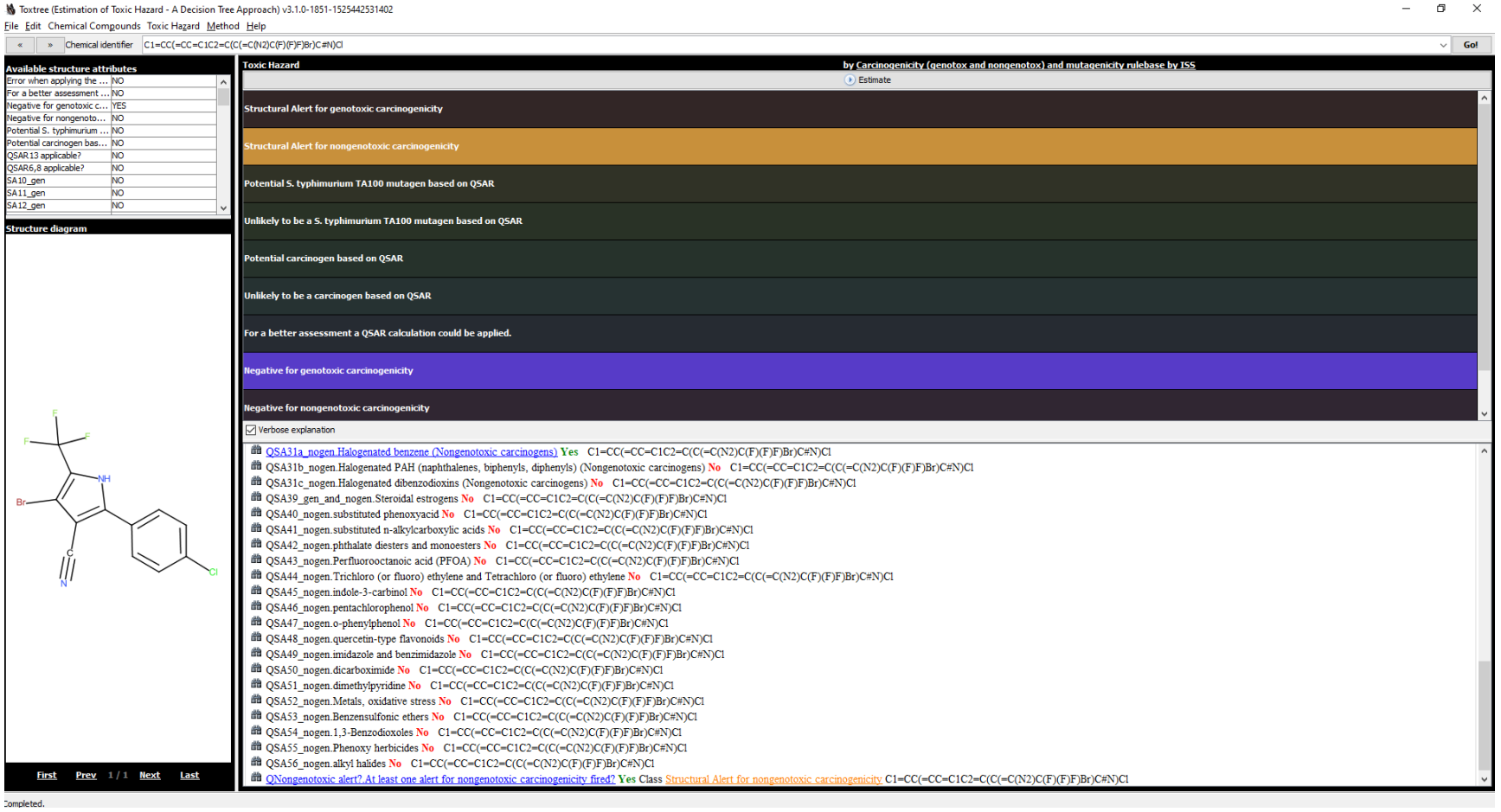
Table 5: Data Gap Assessment Table

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

APPENDIX C: Pharos Output for Tralopyril (CAS #122454-29-9)



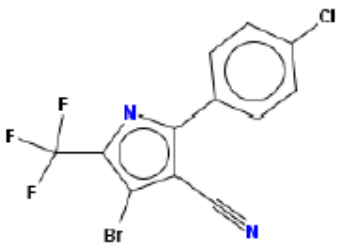
APPENDIX D: Toxtree Carcinogenicity Results for Tralopyril (CAS #122454-29-9)



APPENDIX E: VEGA Carcinogenicity Results for Tralopyril (CAS #122454-29-9)

1. Prediction Summary

Prediction for compound Molecule 0

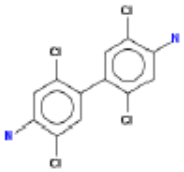


Prediction: Reliability:

Prediction is NON-Carcinogen, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- some similar molecules found in the training set have experimental values that disagree with the predicted value
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (2 infrequent fragments found)
- predicted substance falls into a neuron that is populated by no compounds of the training set

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted Carcinogen activity: NON-Carcinogen
P(Carcinogen): 0.345
P(NON-Carcinogen): 0.655
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

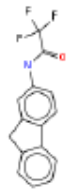
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 15721-02-5
Dataset id: 723 (Training set)
SMILES: Nc1cc(c(cc1Cl)c2cc(c(N)cc2Cl)Cl)Cl
Similarity: 0.743

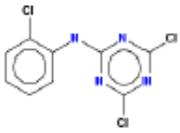
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #2

CAS: 363-17-7
Dataset id: 328 (Training set)
SMILES: O=C(Nc2ccc3c1ccccc1Cc3(c2))C(F)(F)F
Similarity: 0.74

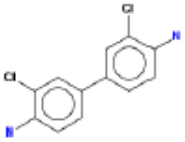
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #3

CAS: 101-05-3
Dataset id: 54 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

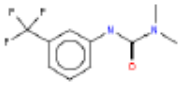
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #4

CAS: 91-94-1
Dataset id: 223 (Training set)
SMILES: Nc1ccc(cc1Cl)c2ccc(N)c(c2)Cl
Similarity: 0.729

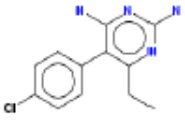
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #5

CAS: 2164-17-2
Dataset id: 327 (Training set)
SMILES: O=C(Nc1cccc(c1)C(F)(F)F)N(C)C
Similarity: 0.727

Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #6

CAS: 58-14-0
Dataset id: 684 (Test set)
SMILES: n1c(nc(c(c1N)c2ccc(cc2)Cl)CC)N
Similarity: 0.725

Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0.308 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.741 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✔	**Accuracy of prediction for similar molecules** Accuracy index = 1 Explanation: accuracy of prediction for similar molecules found in the training set is good.
⚠	**Concordance for similar molecules** Concordance index = 0.501 Explanation: some similar molecules found in the training set have experimental values that disagree with the predicted value.
✔	**Model's descriptors range check** Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (2 infrequent fragments found).
✔	**Model class assignment reliability** Pos/Non-Pos difference = 0.309 Explanation: model class assignment is well defined.
✖	**Neural map neurons concordance** Neurons concordance = 0.5 Explanation: predicted substance falls into a neuron that is populated by no compounds of the training set.

Symbols explanation:

✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.

✖ The feature has a bad assessment, model is not reliable regarding this aspect.

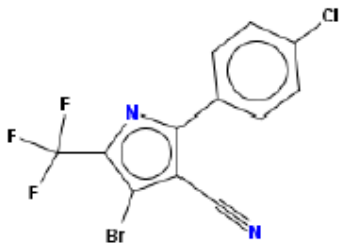
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

	Fragment defined by the SMILES: FC(F)(F)c The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: N#Cc The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

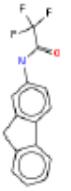


Prediction: Reliability:

Prediction is Carcinogen, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- accuracy of prediction for similar molecules found in the training set is not adequate
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (2 infrequent fragments found)
The following alerts have been found: SA31a Halogenated benzene (Nongenotoxic carcinogens)

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted Carcinogen activity: Carcinogen
Structural alerts: SA31a Halogenated benzene (Nongenotoxic carcinogens)
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

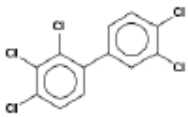
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 363-17-7
Dataset id: 409 (Training set)
SMILES: O=C(Nc2ccc3c1ccccc1Cc3(c2))C(F)(F)F
Similarity: 0.74

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

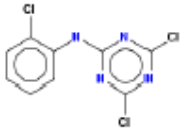


Compound #2

CAS: N.A.
Dataset id: 441 (Training set)
SMILES: c1cc(c(cc1c2ccc(c(c2Cl)Cl)Cl)Cl)Cl
Similarity: 0.739

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): SA31b Halogenated PAH (naphthalenes, biphenyls, diphenyls) (Nongenotoxic carcinogens)

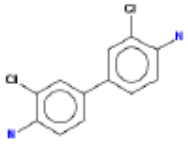


Compound #3

CAS: 101-05-3
Dataset id: 94 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA31a Halogenated benzene (Nongenotoxic carcinogens)

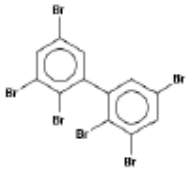


Compound #4

CAS: 91-94-1
Dataset id: 458 (Training set)
SMILES: Nc1ccc(cc1Cl)c2ccc(N)c(c2)Cl
Similarity: 0.729

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): SA28 Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)

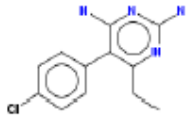


Compound #5

CAS: 67774-32-7
Dataset id: 694 (Training set)
SMILES: c2c(c1cc(cc(c1Br)Br)Br)c(c(cc2Br)Br)Br
Similarity: 0.729

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #6

CAS: 58-14-0
Dataset id: 725 (Training set)
SMILES: n1c(nc(c(c1N)c2ccc(cc2)Cl)CC)N
Similarity: 0.725

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA31a Halogenated benzene (Nongenotoxic carcinogens)

Alerts (not found in the target): SA28 Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0.615 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.739 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.5 Explanation: accuracy of prediction for similar molecules found in the training set is not adequate.
✔	**Concordance for similar molecules** Concordance index = 1 Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (2 infrequent fragments found).

Symbols explanation:

✔ The feature has a good assessment, model is reliable regarding this aspect.

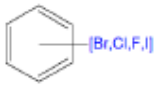
⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.

✖ The feature has a bad assessment, model is not reliable regarding this aspect.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on fragments/structural alerts:

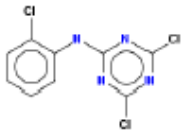
Fragment found: SA31a Halogenated benzene (Nongenotoxic carcinogens)



- Chemicals with two halogens in ortho or meta are excluded.
- Chemicals with three or more hydroxyl groups are excluded.

Halogenated benzene (Nongenotoxic carcinogens). The rule applies only to halogenated benezenes (not naphtalenes, etc.), but it should allow for the presence of other rings in the same molecule. However, the following structures should be excluded:Structures with 2 halogens ortho, Structures with 2 halogens meta, 3 or more hydroxyl groups, 3 or more hydroxyl groups, 3 or more hydroxyl groups, 3 or more hydroxyl groups, 3 or more hydroxyl groups, 3 or more hydroxyl groups with 3 or more hydroxyl groups. Biphenyls, Diphenyls, Not in fused rings

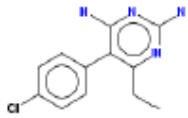
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: 101-05-3
Dataset id: 94 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA31a Halogenated benzene (Nongenotoxic carcinogens)

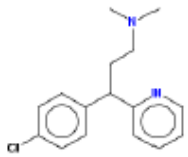


CAS: 58-14-0
Dataset id: 725 (Training set)
SMILES: n1c(nc(c(c1N)c2ccc(cc2)Cl)CC)N
Similarity: 0.725

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA31a Halogenated benzene (Nongenotoxic carcinogens)

Alerts (not found in the target): SA28 Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)



CAS: 113-92-8
Dataset id: 742 (Training set)
SMILES: CN(C)CCC(c1ncccc1)c2ccc(cc2)Cl
Similarity: 0.686

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA31a Halogenated benzene (Nongenotoxic carcinogens)

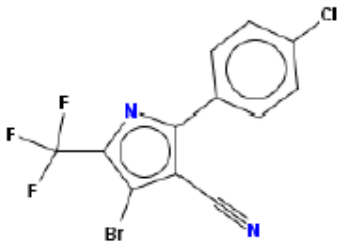
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

	Fragment defined by the SMILES: FC(F)(F)c The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: N#Cc The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

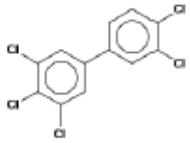


Prediction: Reliability:

Prediction is Possible NON-Carcinogen, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- accuracy of prediction for similar molecules found in the training set is not optimal
- similar molecules found in the training set have experimental values that disagree with the predicted value
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted Mutagen activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 0
Structural alerts: -
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values

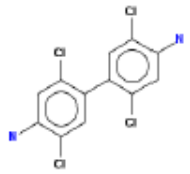


Compound #1

CAS: N.A.
Dataset id: 916 (Training set)
SMILES: c1cc(c(cc1c2cc(c(c(c2)Cl)Cl)Cl)Cl)Cl
Similarity: 0.744

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 97



Compound #2

CAS: N.A.
Dataset id: 727 (Training set)
SMILES: Nc1cc(c(cc1Cl)c2cc(c(N)cc2Cl)Cl)Cl
Similarity: 0.743

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

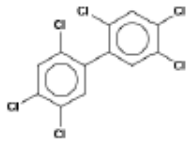
Alerts (not found in the target): Carcinogenity alert no. 23; Carcinogenity alert no. 24

Compound #3

CAS: N.A.
Dataset id: 328 (Training set)
SMILES: O=C(Nc2ccc3c1ccccc1Cc3(c2))C(F)(F)F
Similarity: 0.74

Experimental value: Carcinogen
Predicted value: Carcinogen

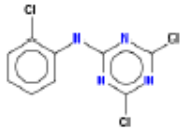
Alerts (not found in the target): Carcinogenity alert no. 22; Carcinogenity alert no. 24; Carcinogenity alert no. 33; Carcinogenity alert no. 125



Compound #4

CAS: N.A.
Dataset id: 866 (Training set)
SMILES: c2c(c1cc(c(cc1Cl)Cl)Cl)c(cc(c2Cl)Cl)Cl
Similarity: 0.734

Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen

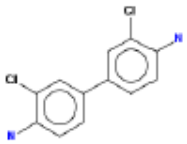


Compound #5

CAS: N.A.
Dataset id: 54 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #6

CAS: N.A.
Dataset id: 223 (Training set)
SMILES: Nc1ccc(cc1Cl)c2ccc(N)c(c2)Cl
Similarity: 0.729

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 23; Carcinogenity alert no. 24

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0.503 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.742 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
⚠	**Accuracy of prediction for similar molecules** Accuracy index = 0.666 Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.
✖	**Concordance for similar molecules** Concordance index = 0.334 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found).

Symbols explanation:

✔ The feature has a good assessment, model is reliable regarding this aspect.
⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.
✖ The feature has a bad assessment, model is not reliable regarding this aspect.

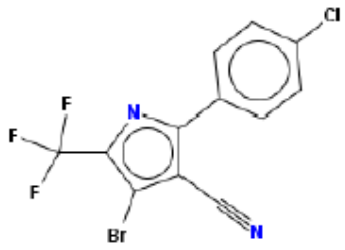
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

Fragment defined by the SMILES: N#Cc
The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

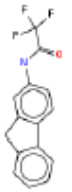


Prediction: Reliability:

Prediction is Possible NON-Carcinogen, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- accuracy of prediction for similar molecules found in the training set is not adequate
- similar molecules found in the training set have experimental values that disagree with the predicted value
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted Mutagen activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 0
Structural alerts: -
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values

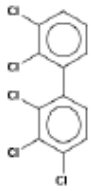


Compound #1

CAS: 363-17-7
Dataset id: 337 (Training set)
SMILES: O=C(Nc2ccc3c1ccccc1Cc3(c2))C(F)(F)F
Similarity: 0.74

Experimental value: Carcinogen
Predicted value: Carcinogen

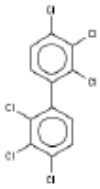
Alerts (not found in the target): Carcinogenity alert no. 36; Carcinogenity alert no. 42



Compound #2

CAS: N.A.
Dataset id: 676 (Training set)
SMILES: c2cc(c1ccc(c(c1Cl)Cl)Cl)c(c(c2)Cl)Cl
Similarity: 0.738

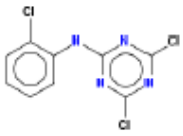
Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #3

CAS: 11096-82-5
Dataset id: 323 (Training set)
SMILES: c1cc(c(c(c1c2ccc(c(c2Cl)Cl)Cl)Cl)Cl)Cl
Similarity: 0.735

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

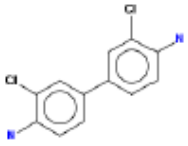


Compound #4

CAS: 101-05-3
Dataset id: 75 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 42



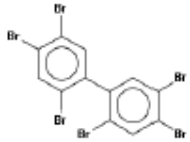
Compound #5

CAS: 91-94-1
Dataset id: 381 (Training set)
SMILES: Nc1ccc(cc1Cl)c2ccc(N)c(c2)Cl
Similarity: 0.729

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 42

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #6

CAS: 67774-32-7
Dataset id: 558 (Training set)
SMILES: c2c(c1cc(c(cc1Br)Br)Br)c(cc(c2Br)Br)Br
Similarity: 0.729

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.738 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.335 Explanation: accuracy of prediction for similar molecules found in the training set is not adequate.
✖	**Concordance for similar molecules** Concordance index = 0 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found).

Symbols explanation:

✔ The feature has a good assessment, model is reliable regarding this aspect.
⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.
✖ The feature has a bad assessment, model is not reliable regarding this aspect.

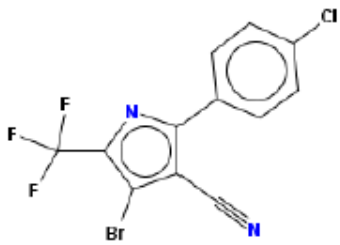
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

Fragment defined by the SMILES: N#Cc
The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

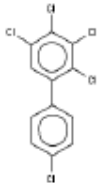


Prediction: Reliability:

Prediction is NON-Carcinogen, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted Oral Carcinogenic class: NON-Carcinogen
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

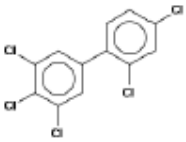
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 74472-37-0
Dataset id: 245 (Test set)
SMILES: c1cc(ccc1c2cc(c(c(c2Cl)Cl)Cl)Cl)Cl
Similarity: 0.74

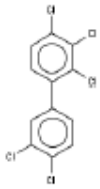
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #2

CAS: 65510-44-3
Dataset id: 247 (Training set)
SMILES: c1cc(cc(c1c2cc(c(c(c2)Cl)Cl)Cl)Cl)Cl
Similarity: 0.74

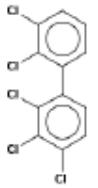
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #3

CAS: 32598-14-4
Dataset id: 244 (Training set)
SMILES: c1cc(c(cc1c2ccc(c(c2Cl)Cl)Cl)Cl)Cl
Similarity: 0.739

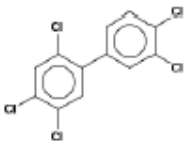
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #4

CAS: 11097-69-1
Dataset id: 23 (Training set)
SMILES: c2cc(c1ccc(c(c1Cl)Cl)Cl)c(c(c2)Cl)Cl
Similarity: 0.738

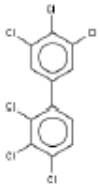
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #5

CAS: 31508-00-6
Dataset id: 246 (Training set)
SMILES: c1cc(c(cc1c2cc(c(cc2Cl)Cl)Cl)Cl)Cl
Similarity: 0.738

Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #6

CAS: 69782-90-7
Dataset id: 165 (Training set)
SMILES: c1cc(c(c(c1c2cc(c(c(c2)Cl)Cl)Cl)Cl)Cl)Cl
Similarity: 0.736

Experimental value: Carcinogen
Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0
Explanation: the predicted compound is outside the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.74
Explanation: only moderately similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 0
Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.

Model's descriptors range check
Descriptors range check = True
Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.

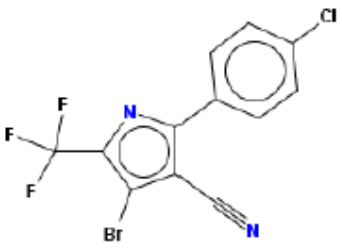
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

1. Prediction Summary

Prediction for compound Molecule 0

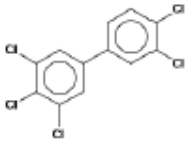


Prediction: Reliability:

Prediction is Carcinogen, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections. Anyway some issues could be not optimal:
- only moderately similar compounds with known experimental value in the training set have been found

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted Inhalation Carcinogenic class: Carcinogen
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

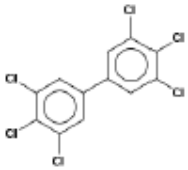
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 57465-28-8
Dataset id: 214 (Training set)
SMILES: c1cc(c(cc1c2cc(c(c(c2)Cl)Cl)Cl)Cl)Cl
Similarity: 0.744

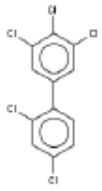
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #2

CAS: 32774-16-6
Dataset id: 139 (Training set)
SMILES: c1c(cc(c(c1Cl)Cl)Cl)c2cc(c(c(c2)Cl)Cl)Cl
Similarity: 0.741

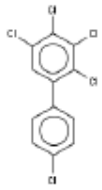
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #3

CAS: 65510-44-3
Dataset id: 210 (Training set)
SMILES: c1cc(cc(c1c2cc(c(c(c2)Cl)Cl)Cl)Cl)Cl
Similarity: 0.74

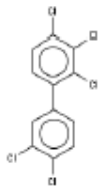
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #4

CAS: 74472-37-0
Dataset id: 213 (Training set)
SMILES: c1cc(ccc1c2cc(c(c(c2Cl)Cl)Cl)Cl)Cl
Similarity: 0.74

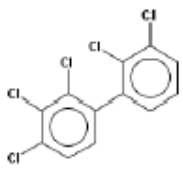
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #5

CAS: 32598-14-4
Dataset id: 212 (Test set)
SMILES: c1cc(c(cc1c2ccc(c(c2Cl)Cl)Cl)Cl)Cl
Similarity: 0.739

Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #6

CAS: 11097-69-1
Dataset id: 21 (Training set)
SMILES: c2cc(c1ccc(c(c1Cl)Cl)Cl)c(c(c2)Cl)Cl
Similarity: 0.738

Experimental value: Carcinogen
Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	**Global AD Index** AD index = 0.862 Explanation: the predicted compound is into the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.742 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✓	**Accuracy of prediction for similar molecules** Accuracy index = 1 Explanation: accuracy of prediction for similar molecules found in the training set is good.
✓	**Concordance for similar molecules** Concordance index = 1 Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.
✓	**Model's descriptors range check** Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.
✓	**Atom Centered Fragments similarity check** ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

✓ The feature has a good assessment, model is reliable regarding this aspect.

⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.

✗ The feature has a bad assessment, model is not reliable regarding this aspect.

APPENDIX F: OncoLogic Output for Tralopyril (CAS #122454-29-9)

EPA OncoLogic 9.0

Target Report

Coded by Oasis Help

Chemical class	Level of concern
Halogenated Aromatic Hydrocarbons	
Halogenated benzenes	Low-moderate

OncoLogic Justification Report

The level of concern for this compound, disregarding any highlighted substituents, is LOW-MODERATE.
The effect of any highlighted substituents is uncertain.

JUSTIFICATION

Halogenated aromatics include the following type of halogenated compounds: benzenes, naphthalenes, biphenyls, terphenyls, diphenyl ethers, diphenyl sulfides, dibenzo-p-dioxins, dibenzofurans, dibenzothiophenes, and diphenyl alkanes and alkenes. Although a number of halogenated aromatics have been shown to be carcinogenic in experimental animals, the mechanism of their carcinogenic action is not clearly understood.

However, there is a prevalent view that these chemicals may be carcinogenic through epigenetic mechanisms rather than by direct action on DNA. For instance, there is considerable evidence showing that the initial event involved in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) carcinogenesis is binding to the cytosolic Ah receptor. The subsequent translocation of the TCDD-receptor complex into the nucleus leads to a modulation of gene expression which is believed to be responsible for the various biochemical (e.g. induction of the cytochrome P-450 1A family) and toxicological effects (including tumorigenesis) of the compound.

Since the key requirement for the binding of TCDD to the cytosolic Ah receptor is a planar molecule with the halogens at the lateral position (i.e., 2,3,7,8-position of TCDD), it has been suggested that other halogenated aromatics with a molecular shape isosteric with TCDD may act by a mechanism similar to that of TCDD. Indeed, like TCDD, a number of halogenated biphenyls and naphthalenes with halogens at the lateral positions are also inducers of the cytochrome P-450 1A family.

Other halogenated biphenyls, naphthalenes and benzenes, which induce the cytochrome P-450 2B family, on the other hand, have been postulated to act via inhibition of "intercellular communication" (also called "metabolic cooperation"). Other epigenetic mechanisms that have been linked to carcinogenesis of halogenated aromatics include (i) hormone imbalance (e.g. estrogen mimics), (ii) immunosuppresion, and (iii) cytotoxicity.

Halogenation of the aromatics renders them more lipid-soluble, more slowly metabolized, and therefore more persistent in animal tissues. In general, the rate of oxidative metabolism decreases as the degree of halogenation increases because of steric hindrance by the halogen atoms. Moreover, the position of halogenation plays an important role in determining the rate of oxidative metabolism. For instance, it has been shown that chlorinated and brominated benzenes having two adjacent unsubstituted carbon atoms are more rapidly metabolized than those without adjacent unsubstituted carbon atoms, despite a similar degree of halogenation. Hence, in addition to the type of halogens, the degree and position of halogenation are important factors in evaluating the carcinogenicity potential of halogenated aromatics.

The carcinogenicity concern levels of these compounds are determined based on structure-activity relationship analysis as well as metabolism and mechanism considerations.

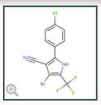
The halogenated benzene with one chloro has a level of concern of LOW-MODERATE.

As a result of the combined substituent modifications, the level of concern remains LOW-MODERATE.

The final level of concern for this compound is LOW-MODERATE.

1 of 1

APPENDIX G: CompTox EDSP21 Results for Tralopyril (CAS #122454-29-9)



Tralopyril

122454-29-9 | DTXSID6041503

Searched by CASRN

Concentration Response Data

Analytical Data on Tox21 Browser

EXPORT

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(4) EDSP AR,EDSP ER,EDSP stero										
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R...	Active				-	cytotoxicity	breast	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R...	Active				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assays use...	ATG_AR_TRANS_up	Inactive				AR	steroidal	liver	cell line
EDSP ER	Estrogen receptor assays used ...	ACEA_ER_80hr	Inactive				ESR1	steroidal	breast	cell line
EDSP ER	Estrogen receptor assays used ...	ATG_ERE_CIS_up	Inactive				ESR1	steroidal	liver	cell line
EDSP ER	Estrogen receptor assays used ...	ACEA_ER_AUC_viability	Active				-	cytotoxicity	breast	cell line
EDSP ER	Estrogen receptor assays used ...	ATG_ERa_TRANS_up	Inactive				ESR1	steroidal	liver	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_CORTIC_dn	Active				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_OHPROG_dn	Active				PGR	progestagens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_ANDR_dn	Inactive				AR	androgens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_OHPROG_up	Inactive				PGR	progestagens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_CORTISOL_dn	Active				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_MTT_cell_viability_dn	Active				-	proliferation	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_ANDR_up	Inactive				AR	androgens	adrenal gland	cell line

Rows: 38 of 558 Total Rows: 558 Filtered: 38

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(4) EDSP AR,EDSP ER,EDSP stero										
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_DOC_up	Inactive				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_MTT_cell_viability_up	Inactive				-	cytotoxicity	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_DOC_dn	Active				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_CORTIC_up	Inactive				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_TESTO_dn	Inactive				AR	androgens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_PROG_dn	Active				PGR	progestagens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_ESTRONE_dn	Inactive				ESR2	estrogens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_11DCORT_up	Inactive				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_TESTO_up	Inactive				AR	androgens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_ESTRADIOL_dn	Inactive				ESR2	estrogens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_ESTRONE_up	Inactive				ESR2	estrogens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_CORTISOL_up	Inactive				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_OHPREG_up	Inactive				PGR	progestagens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_ESTRADIOL_up	Inactive				ESR2	estrogens	adrenal gland	cell line

Rows: 38 of 558 Total Rows: 558 Filtered: 38

EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_11DCORT_dn	Active				NR3C1	glucocorticoids	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_OHPREG_dn	Inactive				PGR	progestagens	adrenal gland	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	CEETOX_H295R_PROG_up	Inactive				PGR	progestagens	adrenal gland	cell line
EDSP thyroid	Thyroid pathway assays used in...	TOX21_TRHR_HEK293_Antagonist	Inactive				TRHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used in...	TOX21_TSHR_HTRF_Agonist_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used in...	TOX21_TSHR_HTRF_wt_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used in...	TOX21_TRHR_HEK293_Agonist	Inactive				TRHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used in...	ATG_THRa1_TRANS_dn	Inactive				THRA	non-steroidal	liver	cell line
EDSP thyroid	Thyroid pathway assays used in...	TOX21_TSHR_HTRF_Antagonist_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used in...	ATG_THRa1_TRANS_up	Active				THRA	non-steroidal	liver	cell line

Rows: 38 of 558 Total Rows: 558 Filtered: 38

APPENDIX H: Danish QSAR Endocrine Results for Tralopyril (CAS #122454-29-9)

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		NEG_OUT	INC_OUT	NEG_IN	INC_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		INC_OUT	INC_OUT	POS_IN	NEG_IN
Estrogen Receptor α Activation (Human *in vitro*)		NEG_IN	INC_OUT	NEG_IN	NEG_IN
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human *in vitro*)		NEG_IN	POS_OUT	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (*in vitro*)		N/A	N/A	NEG_OUT	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	NEG_OUT	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Sodium/iodide symporter (NIS), higher sensitivity		N/A	N/A	POS_IN	N/A
Sodium/iodide symporter (NIS), higher specificity		N/A	N/A	POS_IN	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L			55910.95	1438.026	357.5996
- µM			159955.8	4114.054	1023.058
- Positive for IC_{50} ≤ 10 µM					
- Positive for IC_{50} ≤ 100 µM					
- Domain		OUT	OUT	OUT	OUT
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L			11310.9	43.86689	79.6337
- µM			32359.38	125.4989	227.8243
- Positive for IC_{50} ≤ 10 µM					
- Positive for IC_{50} ≤ 100 µM					
- Domain		OUT	OUT	OUT	OUT
Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	POS_IN	N/A
Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	POS_IN	N/A
Pregnane X Receptor (PXR) Binding (Human *in vitro*)	N/A	POS_IN	POS_OUT	POS_IN	POS_IN

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, without OH or NH2 group
- metabolites from *in vivo* Rat metabolism simulator only	Strong binder, OH group
- metabolites from Rat liver S9 metabolism simulator only	Strong binder, OH group
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found

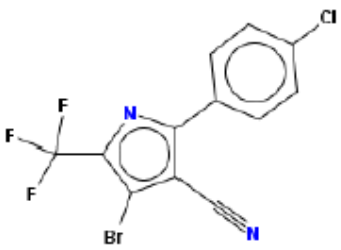
OECD QSAR Toolbox v.4.2 profilers

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

APPENDIX I: VEGA Endocrine Results for Tralopyril (CAS #122454-29-9)

1. Prediction Summary

Prediction for compound Molecule 0

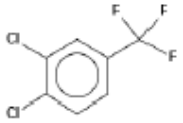


Prediction: Reliability:

Prediction is Active, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- similar molecules found in the training set have experimental values that disagree with the predicted value
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (2 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted activity: Active
Classification tree final node: 18
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

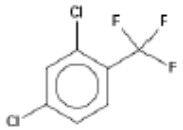
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 328-84-7
Dataset id: 292 (Test set)
SMILES: FC(F)(F)c1ccc(c(c1)Cl)Cl
Similarity: 0.728

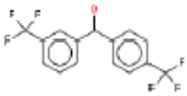
Experimental value: Inactive
Predicted value: Inactive



Compound #2

CAS: 320-60-5
Dataset id: 294 (Training set)
SMILES: FC(F)(F)c1ccc(cc1Cl)Cl
Similarity: 0.727

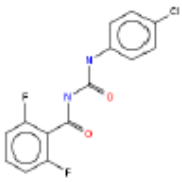
Experimental value: Inactive
Predicted value: Inactive



Compound #3

CAS: 21084-22-0
Dataset id: 669 (Test set)
SMILES: O=C(c1ccc(cc1)C(F)(F)F)c2cccc(c2)C(F)(F)F
Similarity: 0.726

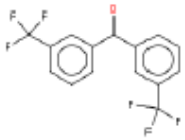
Experimental value: Inactive
Predicted value: Inactive



Compound #4

CAS: 35367-38-5
Dataset id: 617 (Training set)
SMILES: O=C(NC(=O)c1c(F)cccc1(F))Nc2ccc(cc2)Cl
Similarity: 0.725

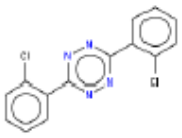
Experimental value: Inactive
Predicted value: Inactive



Compound #5

CAS: 1868-00-4
Dataset id: 665 (Training set)
SMILES: O=C(c1cccc(c1)C(F)(F)F)c2cccc(c2)C(F)(F)F
Similarity: 0.725

Experimental value: Inactive
Predicted value: Inactive



Compound #6

CAS: 74115-24-5
Dataset id: 856 (Training set)
SMILES: n1nc(nnc1c2ccccc2Cl)c3ccccc3Cl
Similarity: 0.722

Experimental value: Inactive
Predicted value: Inactive

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.727 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✔	**Accuracy of prediction for similar molecules** Accuracy index = 1 Explanation: accuracy of prediction for similar molecules found in the training set is good.
✖	**Concordance for similar molecules** Concordance index = 0 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✔	**Model's descriptors range check** Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (2 infrequent fragments found).

Symbols explanation:

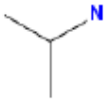
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.

✖ The feature has a bad assessment, model is not reliable regarding this aspect.

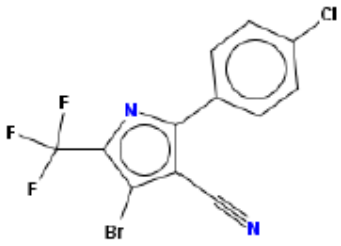
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

	Fragment defined by the SMILES: cc(c)n The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: N#Cc The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

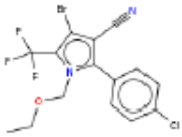


Prediction: Reliability:

Prediction is NON-active, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections. Anyway some issues could be not optimal:
- only moderately similar compounds with known experimental value in the training set have been found
The following relevant fragments have been found: ER non-activity alert no. 30; ER possible non-activity alert no. 8

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted ER-mediated effect: NON-active
No. alerts for activity: 0
No. alerts for possible activity: 0
No. alerts for non-activity: 1
No. alerts for possible non-activity: 1
Structural alerts: ER non-activity alert no. 30; ER possible non-activity alert no. 8
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values

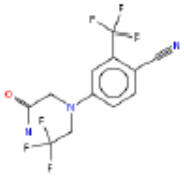


Compound #1

CAS: N.A.
Dataset id: 1408 (Training set)
SMILES: N#Cc2c(c1ccc(cc1)Cl)n(c(c2Br)C(F)(F)F)COCC
Similarity: 0.905

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 30; ER possible non-activity alert no. 8

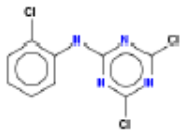


Compound #2

CAS: N.A.
Dataset id: 1527 (Training set)
SMILES: N#Cc1ccc(cc1C(F)(F)F)N(CC(=O)N)CC(F)(F)F
Similarity: 0.732

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 4; ER non-activity alert no. 25; ER possible non-activity alert no. 4; ER possible non-activity alert no. 7; ER possible non-activity alert no. 9



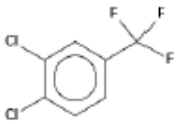
Compound #3

CAS: N.A.
Dataset id: 350 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER possible non-activity alert no. 8

Alerts (not found in the target): ER non-activity alert no. 9; ER possible non-activity alert no. 4



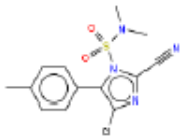
Compound #4

CAS: N.A.
Dataset id: 715 (Training set)
SMILES: FC(F)(F)c1ccc(c(c1)Cl)Cl
Similarity: 0.728

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER possible non-activity alert no. 8

Alerts (not found in the target): ER non-activity alert no. 15



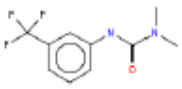
Compound #5

CAS: N.A.
Dataset id: 1405 (Training set)
SMILES: N#Cc2nc(c(c1ccc(cc1)C)n2S(=O)(=O)N(C)C)Cl
Similarity: 0.728

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 21

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #6

CAS: N.A.
Dataset id: 962 (Training set)
SMILES: O=C(Nc1cccc(c1)C(F)(F)F)N(C)C
Similarity: 0.727

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 10; ER non-activity alert no. 19; ER possible non-activity alert no. 4; ER possible non-activity alert no. 9

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	**Global AD Index** AD index = 0.875 Explanation: the predicted compound is into the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.765 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✓	**Accuracy of prediction for similar molecules** Accuracy index = 1 Explanation: accuracy of prediction for similar molecules found in the training set is good.
✓	**Concordance for similar molecules** Concordance index = 1 Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.
✓	**Atom Centered Fragments similarity check** ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

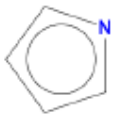
Symbols explanation:

✓ The feature has a good assessment, model is reliable regarding this aspect.
⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.
✗ The feature has a bad assessment, model is not reliable regarding this aspect.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

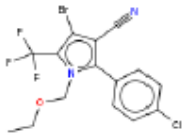
(Molecule 0) Reasoning on fragments/structural alerts - 1 of 2:

Fragment found: ER non-activity alert no. 30



Fragment related to non-activity for ER-mediated effect, defined by the SMARTS: n1cccc1

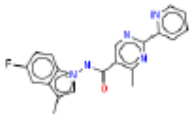
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: N.A.
Dataset id: 1408 (Training set)
SMILES: N#Cc2c(c1ccc(cc1)Cl)n(c(c2Br)C(F)(F)F)COCC
Similarity: 0.905

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 30; ER possible non-activity alert no. 8

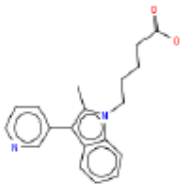


CAS: N.A.
Dataset id: 1529 (Training set)
SMILES: O=C(Nn2cc(c1cc(F)ccc12)C)c3cnc(nc3C)c4ncccc4
Similarity: 0.69

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 30

Alerts (not found in the target): ER non-activity alert no. 3; ER non-activity alert no. 11; ER possible non-activity alert no. 9



CAS: N.A.
Dataset id: 1454 (Training set)
SMILES: O=C(O)CCCCn3c1ccccc1c(c2cnccc2)c3C
Similarity: 0.664

Experimental value: NON-active
Predicted value: NON-active

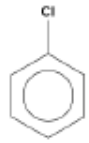
Alerts (found also in the target): ER non-activity alert no. 30

Alerts (not found in the target): ER non-activity alert no. 3; ER possible non-activity alert no. 2; ER possible non-activity alert no. 9

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

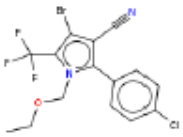
(Molecule 0) Reasoning on fragments/structural alerts - 2 of 2:

Fragment found: ER possible non-activity alert no. 8



Fragment related to possible non-activity for ER-mediated effect, defined by the SMARTS: c1ccccc1Cl

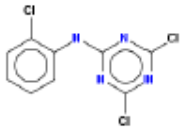
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: N.A.
Dataset id: 1408 (Training set)
SMILES: N#Cc2c(c1ccc(cc1)Cl)n(c(c2Br)C(F)(F)F)COCC
Similarity: 0.905

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 30; ER possible non-activity alert no. 8

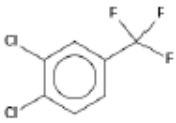


CAS: N.A.
Dataset id: 350 (Training set)
SMILES: n1c(nc(nc1Cl)Cl)Nc2ccccc2Cl
Similarity: 0.73

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER possible non-activity alert no. 8

Alerts (not found in the target): ER non-activity alert no. 9; ER possible non-activity alert no. 4



CAS: N.A.
Dataset id: 715 (Training set)
SMILES: FC(F)(F)c1ccc(c(c1)Cl)Cl
Similarity: 0.728

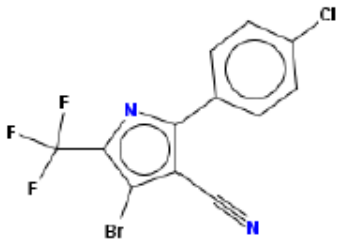
Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER possible non-activity alert no. 8

Alerts (not found in the target): ER non-activity alert no. 15

1. Prediction Summary

Prediction for compound Molecule 0

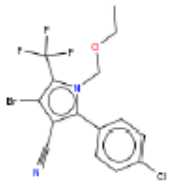


Prediction: Reliability:

Prediction is NON-active, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections.

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted AR binding activity: NON-active
No. alerts for binding activity: 0
No. alerts for non-binding activity: 0
Structural alerts: -
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

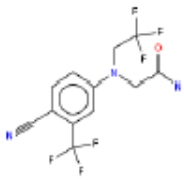
CAS: 122453-73-0
Dataset id: 1602 (Training set)
SMILES: CCOC[n]1c(c(C#N)c(Br)c1C(F)(F)F)-c1ccc(Cl)cc1
Similarity: 0.905

Experimental value: NON-active
Predicted value: NON-active

Compound #2

CAS: 131341-86-1
Dataset id: 1061 (Training set)
SMILES: N#Cc1c[nH]cc1-c1cccc2OC(F)(F)Oc21
Similarity: 0.775

Experimental value: NON-active
Predicted value: NON-active

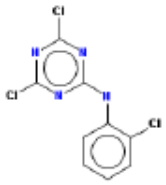


Compound #3

CAS: 864283-48-7
Dataset id: 205 (Training set)
SMILES: NC(=O)CN(CC(F)(F)F)c1cc(c(cc1)C#N)C(F)(F)F
Similarity: 0.732

Experimental value: Active
Predicted value: Active

Alerts (not found in the target): ER alert no. 8, active; ER alert no. 127, active

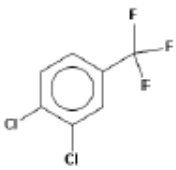


Compound #4

CAS: 101-05-3
Dataset id: 262 (Training set)
SMILES: Clc1ccccc1Nc1[n]c(Cl)[n]c(Cl)[n]1
Similarity: 0.73

Experimental value: NON-active
Predicted value: NON-active

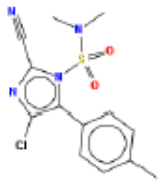
Alerts (not found in the target): ER alert no. 22, inactive



Compound #5

CAS: 328-84-7
Dataset id: 938 (Training set)
SMILES: FC(F)(F)c1cc(Cl)c(Cl)cc1
Similarity: 0.728

Experimental value: NON-active
Predicted value: NON-active



Compound #6

CAS: 120116-88-3
Dataset id: 1608 (Training set)
SMILES: CN(C)S(=O)(=O)[n]1c(c(Cl)[n]c1C#N)-c1ccc(C)cc1
Similarity: 0.728

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER alert no. 101, inactive

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.909
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.826
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 1
Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.

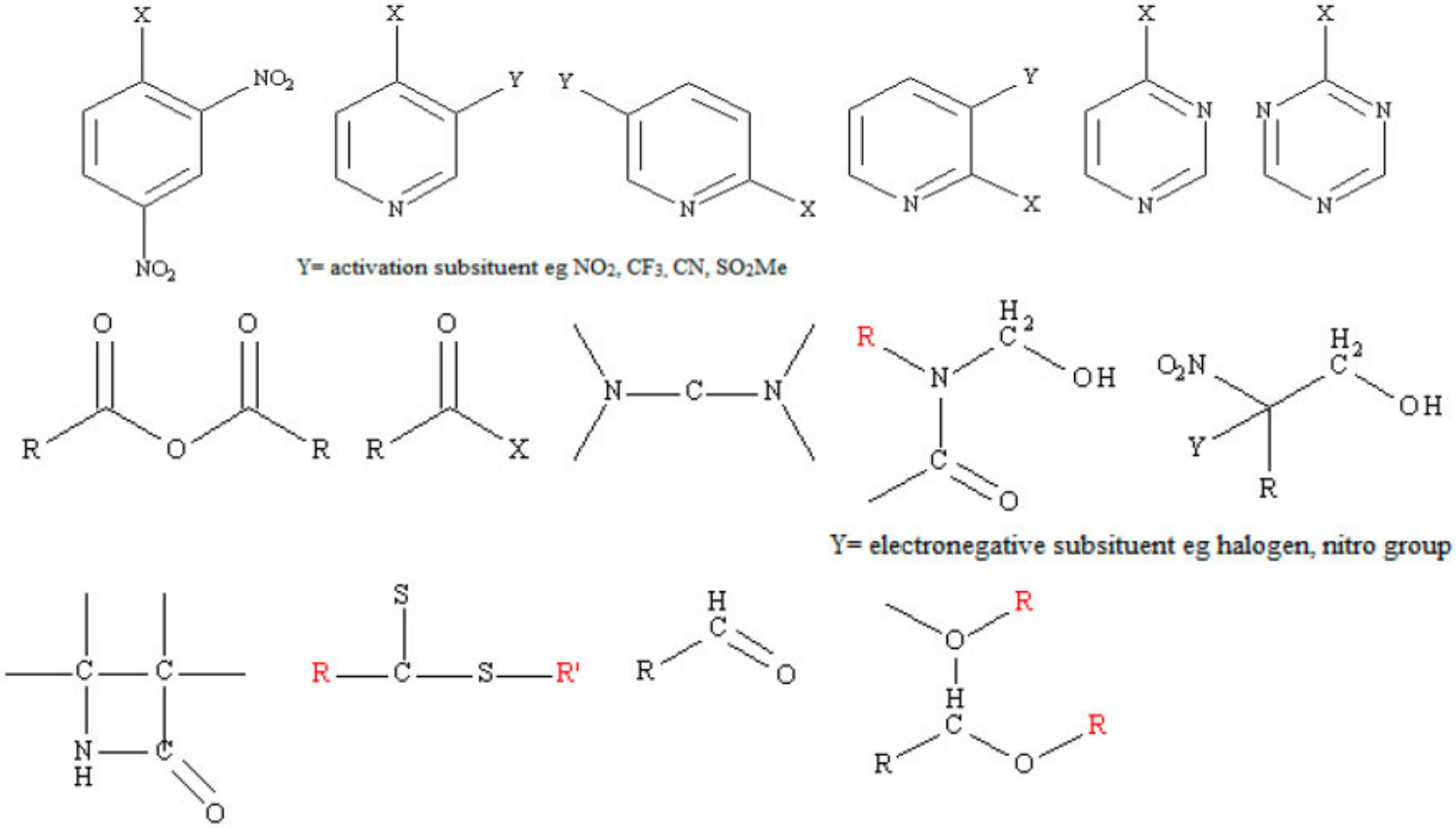
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

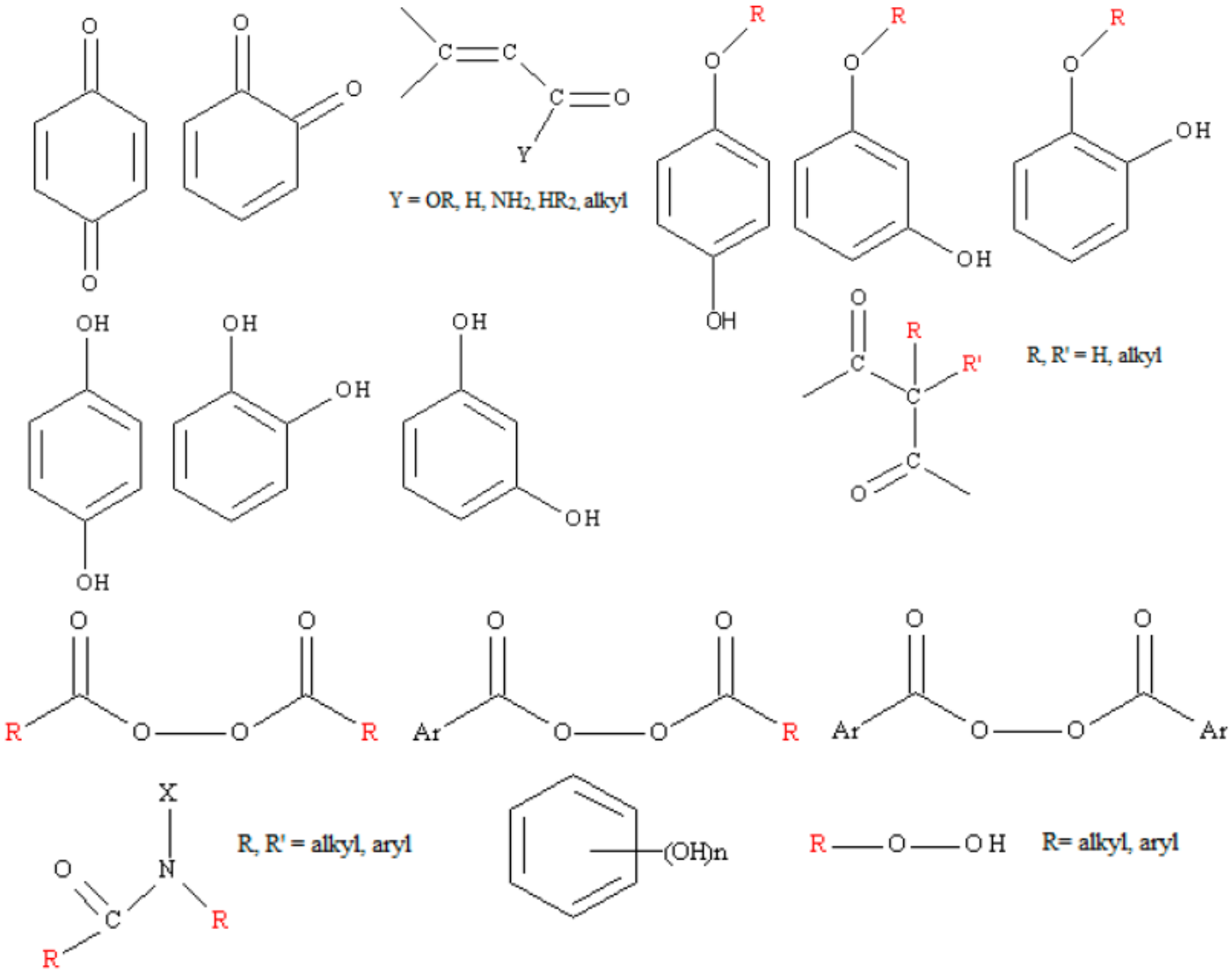
Symbols explanation:

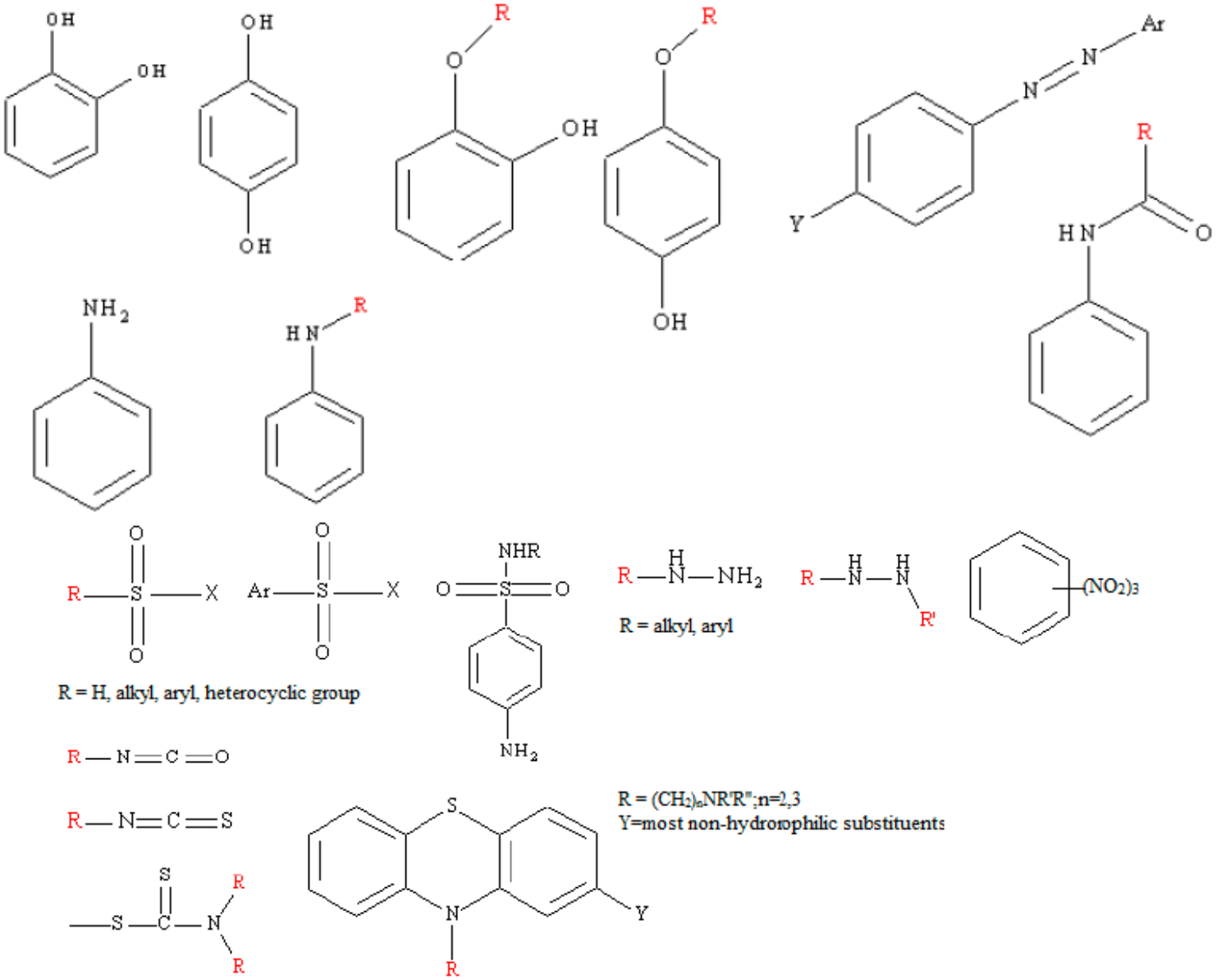
The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

APPENDIX J: Known Structural Alerts for Skin Sensitization

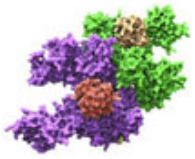
Below are known structural alerts for skin sensitizers (Payne and Walsh 1994). Tralopyril possesses no known structural alerts for skin sensitization.

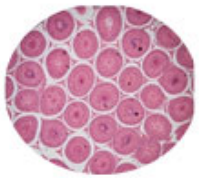






APPENDIX K: LabMol Skin Sensitization Modeling Results for Tralopyril (CAS #122454-29-9)

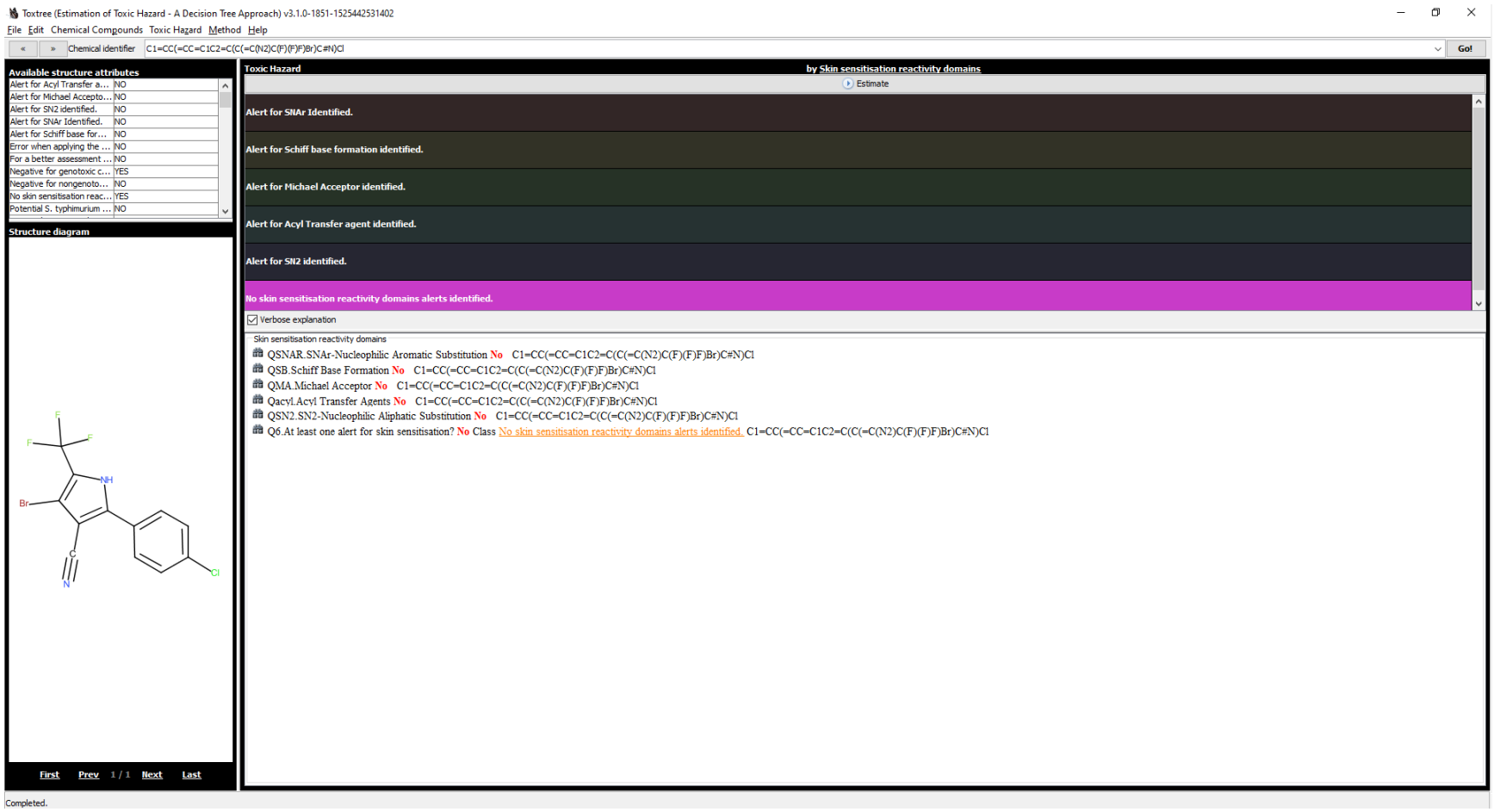





Chemical Exposure	Molecular initiating event *in chemico*	Cellular response *in vitro*		Tissue / Organ response *in vivo*	Organism response *in vivo*	Pred-Skin 3.0 Outcome *in silico*
•Skin Penetration •Electrophilic substance: directly or via auto-oxidation or metabolism	Covalent interaction with proteins in the skin (OECD442C) Haptenation: covalent modification of epidermal proteins	Keratinocyte responses (OECD442D) • Activation of inflammatory cytokines •Induce cytoprotective genes	Dendritic cells (DCs) (OECD442E) • Induction of inflammatory cytokines •Mobilization of DCs	Proliferation of antigen-specific T cells (OECD429) •Histocompatibility complex representation by DCs •Activation of T cells •Proliferation of activated T cells	Inflammation upon challenge allergen To maximise the use of existing knowledge, we also incorporate historical HRIPT (human repeated insult patch test) and HMT (human maximization test)	The Bayesian model is a consensus model integrating predictions from all the other assays for an integrative qualitative risk assessment (QRA) of skin sensitization based on the weight of evidence (WoE).
•Exposure consideration ? •Physicochemical and Biopharmaceutical properties ? •Skin Penetration ? •Skin Metabolism ?	**Prediction DPRA** Sensitizer (+) (AD, Confiability) (Outside , 87.3%) Probability map	**Prediction KeratinoSens** Non-Sensitizer (-) (AD, Confiability) (Outside, 82.1%) Probability map	**Prediction h-CLAT** Sensitizer (+) (AD, Confiability) (Inside, 62.0%) Probability map	**Prediction LLNA** Sensitizer (+) (AD, Confiability) (Outside, 61.8%) Probability map	**Prediction HRIPT/HMT** Sensitizer (+) (AD, Confiability) (Outside, 59.9%) Probability map	**Bayesian Outcome** Sensitizer (+) (Confiability) (High)

Low (-) confidence prediction for the Bayesian model means two or more individual predictions are in disagreement with Bayesian Outcome.

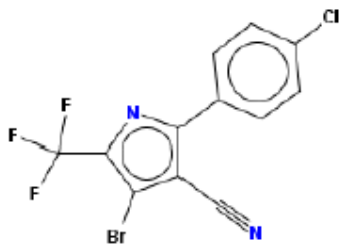
APPENDIX L: Toxtree Skin Sensitization Results for Tralopyril (CAS #122454-29-9)



APPENDIX M: VEGA Skin Sensitization Results for Tralopyril (CAS #122454-29-9)

1. Prediction Summary

Prediction for compound Molecule 0

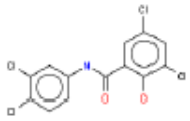


Prediction: Reliability:

Prediction is Sensitizer, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- accuracy of prediction for similar molecules found in the training set is not adequate
- a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (8 unknown fragments and 1 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted skin sensitization activity: Sensitizer
O(Active): 1
O(Inactive): 0
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

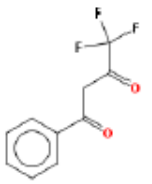
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 1154-59-2
Dataset id: 197 (Training set)
SMILES: O=C(Nc1ccc(c(c1)Cl)Cl)c2cc(cc(c2(O))Cl)Cl
Similarity: 0.711

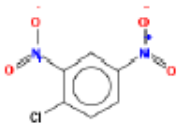
Experimental value: Sensitizer
Predicted value: Sensitizer



Compound #2

CAS: 326-06-7
Dataset id: 203 (Training set)
SMILES: O=C(c1ccccc1)CC(=O)C(F)(F)F
Similarity: 0.689

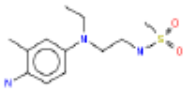
Experimental value: Sensitizer
Predicted value: NON-Sensitizer



Compound #3

CAS: 97-00-7
Dataset id: 55 (Training set)
SMILES: O=[N+]([O-])c1ccc(c(c1)[N+](=O)[O-])Cl
Similarity: 0.653

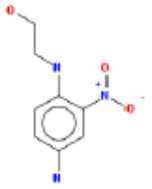
Experimental value: Sensitizer
Predicted value: Sensitizer



Compound #4

CAS: N.A.
Dataset id: 92 (Training set)
SMILES: O=S(=O)(NCCN(c1ccc(N)c(c1)C)CC)C
Similarity: 0.647

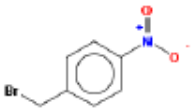
Experimental value: Sensitizer
Predicted value: Sensitizer



Compound #5

CAS: 2871-01-4
Dataset id: 5 (Training set)
SMILES: O=[N+]([O-])c1cc(N)ccc1(NCCO)
Similarity: 0.646

Experimental value: Sensitizer
Predicted value: Sensitizer



Compound #6

CAS: 100-11-8
Dataset id: 163 (Training set)
SMILES: O=[N+]([O-])c1ccc(cc1)CBr
Similarity: 0.645

Experimental value: Sensitizer
Predicted value: Sensitizer

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0.241 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.7 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.512 Explanation: accuracy of prediction for similar molecules found in the training set is not adequate.
✔	**Concordance for similar molecules** Concordance index = 1 Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.
✔	**Model's descriptors range check** Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.
✖	**Atom Centered Fragments similarity check** ACF index = 0.34 Explanation: a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (8 unknown fragments and 1 infrequent fragments found).

Symbols explanation:

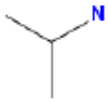
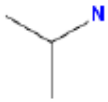
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.

✖ The feature has a bad assessment, model is not reliable regarding this aspect.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments - page 1 of 2.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

	Fragment defined by the SMILES: FC The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: cc(c)c The fragment has never been found in the model's training set
	Fragment defined by the SMILES: cc(c)n The fragment has never been found in the model's training set
	Fragment defined by the SMILES: cc(c)Br The fragment has never been found in the model's training set
	Fragment defined by the SMILES: cc(n)C The fragment has never been found in the model's training set
	Fragment defined by the SMILES: FC(F)(F)c The fragment has never been found in the model's training set
	Fragment defined by the SMILES: cBr The fragment has never been found in the model's training set

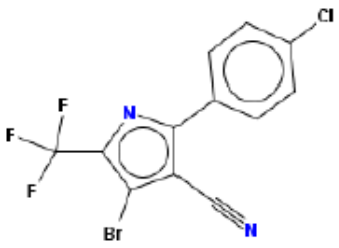
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments - page 2 of 2.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

	Fragment defined by the SMILES: N#Cc The fragment has never been found in the model's training set
	Fragment defined by the SMILES: N#C The fragment has never been found in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

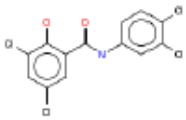


Prediction: Reliability:

Prediction is NON-Sensitizer, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- accuracy of prediction for similar molecules found in the training set is not adequate
- similar molecules found in the training set have experimental values that disagree with the predicted value
- 1 descriptor(s) for this compound have values outside the descriptor range of the compounds of the training set.
- a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (5 unknown fragments and 3 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#Cc1c([nH]c(c1Br)C(F)(F)F)c2ccc(cc2)Cl
Experimental value: -
Predicted skin sensitization activity: NON-Sensitizer
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

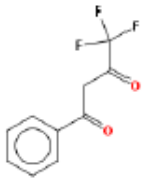
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 1154-59-2
Dataset id: 77 (Training set)
SMILES: O=C(Nc1ccc(c(c1)Cl)Cl)c2cc(cc(c2(O))Cl)Cl
Similarity: 0.711

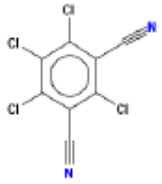
Experimental value: Sensitizer
Predicted value: Sensitizer



Compound #2

CAS: 326-06-7
Dataset id: 130 (Training set)
SMILES: O=C(c1ccccc1)CC(=O)C(F)(F)F
Similarity: 0.689

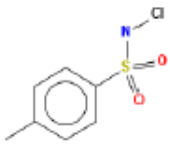
Experimental value: Sensitizer
Predicted value: NON-Sensitizer



Compound #3

CAS: 1897-45-6
Dataset id: 151 (Training set)
SMILES: N#Cc1c(c(C#N)c(c(c1Cl)Cl)Cl)Cl
Similarity: 0.683

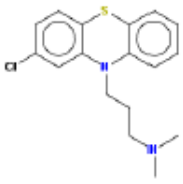
Experimental value: Sensitizer
Predicted value: NON-Sensitizer



Compound #4

CAS: 127-65-1
Dataset id: 303 (Test set)
SMILES: O=S(=O)(NCl)c1ccc(cc1)C
Similarity: 0.655

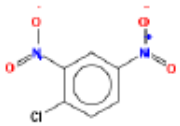
Experimental value: Sensitizer
Predicted value: NON-Sensitizer



Compound #5

CAS: 69-09-0
Dataset id: 152 (Training set)
SMILES: c1ccc3c(c1)N(c2cc(ccc2S3)Cl)CCCN(C)C
Similarity: 0.654

Experimental value: Sensitizer
Predicted value: NON-Sensitizer



Compound #6

CAS: 97-00-7
Dataset id: 16 (Training set)
SMILES: O=[N+]([O-])c1ccc(c(c1)[N+](=O)[O-])Cl
Similarity: 0.653

Experimental value: Sensitizer
Predicted value: Sensitizer

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.7 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.512 Explanation: accuracy of prediction for similar molecules found in the training set is not adequate.
✖	**Concordance for similar molecules** Concordance index = 0 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✖	**Model's descriptors range check** Descriptors range check = False Explanation: 1 descriptor(s) for this compound have values outside the descriptor range of the compounds of the training set..
✖	**Atom Centered Fragments similarity check** ACF index = 0.28 Explanation: a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (5 unknown fragments and 3 infrequent fragments found).

Symbols explanation:

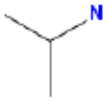
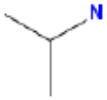
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.

✖ The feature has a bad assessment, model is not reliable regarding this aspect.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments - page 1 of 2.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

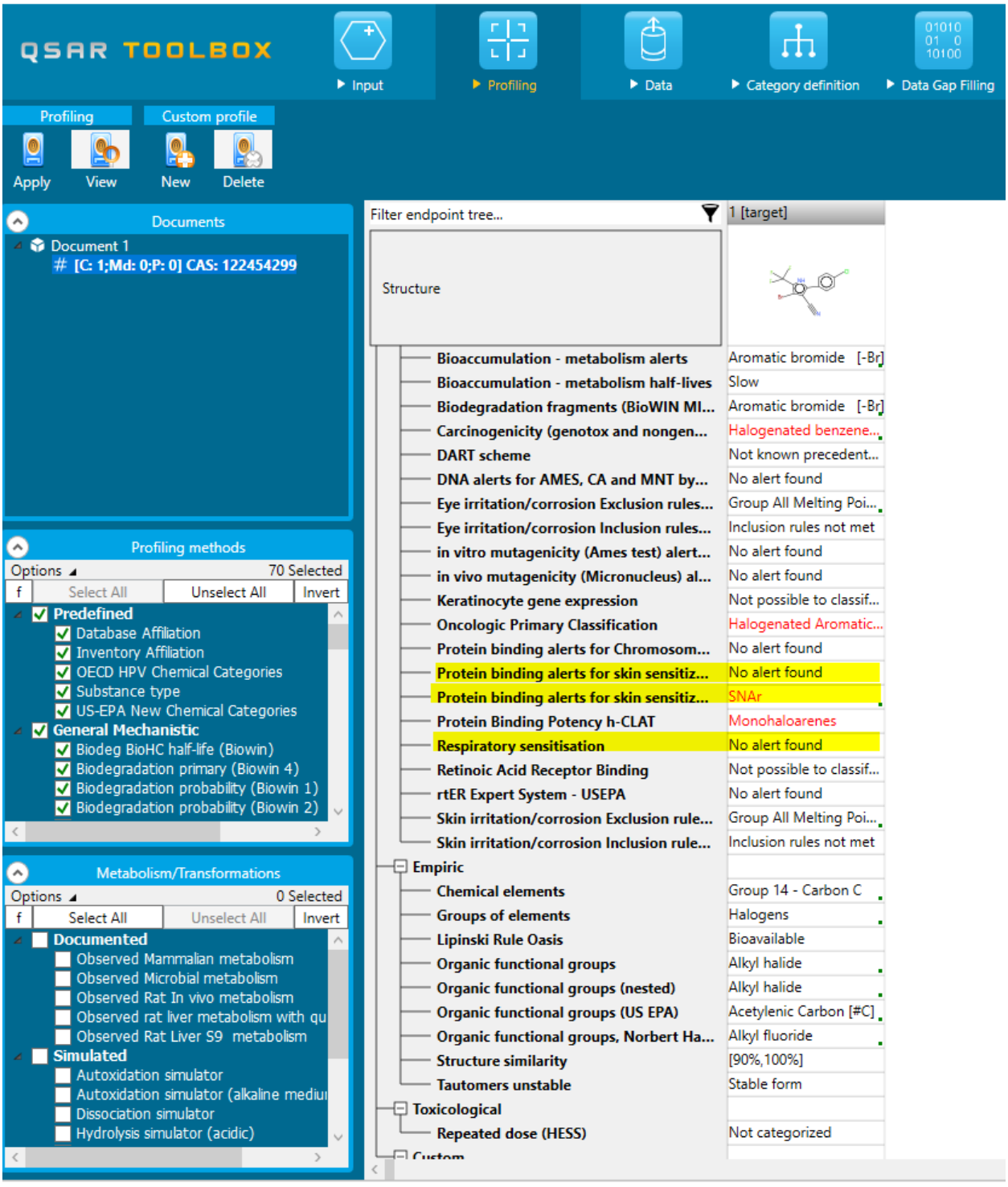
	Fragment defined by the SMILES: cc(n)C The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: FC The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: N#Cc The fragment has less than 3 occurrences in the model's training set
	Fragment defined by the SMILES: cc(c)c The fragment has never been found in the model's training set
	Fragment defined by the SMILES: cc(c)n The fragment has never been found in the model's training set
	Fragment defined by the SMILES: cc(c)Br The fragment has never been found in the model's training set
	Fragment defined by the SMILES: FC(F)(F)c The fragment has never been found in the model's training set

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments - page 2 of 2.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:

Fragment defined by the SMILES: cBr
The fragment has never been found in the model's training set

APPENDIX N: OECD Toolbox Skin and Respiratory Sensitization Results for Tralopyril (CAS #122454-29-9)



APPENDIX O: Danish QSAR Skin and Respiratory Sensitization Results for Tralopyril (CAS #122454-29-9)

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Severe Skin Irritation in Rabbit		NEG_OUT	INC_OUT	INC_OUT	NEG_IN
Skin sensitisation GHS/CLP at least Cat. 1, LLNA-based (open data only)				INC_OUT	
Skin sensitisation GHS/CLP at least Cat. 1, LLNA-based (open data and REACH-registrations)	N/A			NEG_IN	
Skin sensitisation GHS/CLP at least Cat. 1, LLNA-based, only negative predictions (open data only)				N/A	
Skin sensitisation GHS/CLP Cat. 1A, LLNA-based (open data only)				INC_OUT	
Skin sensitisation GHS/CLP Cat. 1A, LLNA-based (open data and REACH-registrations)	N/A			NEG_IN	
Skin sensitisation GHS/CLP Cat. 1A, LLNA-based, only positive predictions (open data and REACH-registrations)	N/A			N/A	
Allergic Contact Dermatitis in Guinea Pig and Human*	N/A	INC_OUT	INC_OUT	POS_OUT	INC_OUT
Respiratory Sensitisation in Humans		INC_OUT	INC_OUT	POS_OUT	POS_OUT

DTU-developed models

**Based on commercial training set*

Protein binding by OASIS, alerts in:	
- parent only	Halogenated five membered aromatic compounds
- metabolites from skin metabolism simulator only	
- metabolites from auto-oxidation simulator only	
Protein binding by OECD, alerts in:	
- parent only	No alert found
- metabolites from skin metabolism simulator only	
- metabolites from auto-oxidation simulator only	
Protein binding potency Cys (DRPA 13%), alerts in:	
- parent only	DPRA less than 9% (DPRA 13%) >> Mono-halo arenes
- metabolites from skin metabolism simulator only	
- metabolites from auto-oxidation simulator only	
Protein binding potency Lys (DRPA 13%), alerts in:	
- parent only	DPRA less than 9% (DPRA 13%) >> Mono-halo arenes
- metabolites from skin metabolism simulator only	
- metabolites from auto-oxidation simulator only	
Keratinocyte gene expression, alerts in:	
- parent only	Not possible to classify according to these rules
- metabolites from skin metabolism simulator only	
- metabolites from auto-oxidation simulator only	
Protein binding potency GSH, alerts in:	
- parent only	Not possible to classify according to these rules (GSH)

OECD QSAR Toolbox v.4.1 profilers

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

APPENDIX P: EPI Suite™ Modeling Results for Tralopyril (CAS #122454-29-9)

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

CAS Number:
SMILES : c1cc(ccc1c2c(c(c(n2)C(F)(F)F)Br)C(#N))CL
CHEM :
MOL FOR: C12 H5 Br1 CL1 F3 N2
MOL WT : 349.54
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 3.50
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 v1.69 estimate) = 4.69

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 396.22 (Adapted Stein & Brown method)
Melting Pt (deg C): 151.82 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 5.24E-007 (Modified Grain method)
VP (Pa, 25 deg C) : 6.98E-005 (Modified Grain method)
Subcooled liquid VP: 1.03E-005 mm Hg (25 deg C, Mod-Grain method)
: 0.00137 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 3.474
log Kow used: 3.50 (user entered)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.067762 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found: Pyrazoles/Pyrroles

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 1.73E-008 atm-m3/mole (1.75E-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: 6.937E-008 atm-m3/mole (7.029E-003 Pa-m3/mole)
VP: 5.24E-007 mm Hg (source: MPBPVP)
WS: 3.47 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 3.50 (user entered)
Log Kaw used: -6.150 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 9.650
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.0750
Biowin2 (Non-Linear Model) : 0.0013
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 1.4888 (recalcitrant)
Biowin4 (Primary Survey Model) : 2.6850 (weeks-months)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.2298
Biowin6 (MITI Non-Linear Model): 0.0000
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.0521
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): 0.00137 Pa (1.03E-005 mm Hg)
Log Koa (Koawin est): 9.650
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.00218
Octanol/air (Koa) model: 0.0011
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.0731
Mackay model : 0.149
Octanol/air (Koa) model: 0.0806

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 1.6564 E-12 cm3/molecule-sec
Half-Life = 6.457 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 77.487 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Reaction With Nitrate Radicals May Be Important!
Fraction sorbed to airborne particulates (phi):
0.111 (Junge-Pankow, Mackay avg)
0.0806 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 3.477E+004 L/kg (MCI method)
Log Koc: 4.541 (MCI method)

Koc : 1792 L/kg (Kow method)
Log Koc: 3.253 (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 = 1.976 (BCF = 94.69 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.8479 days (HL = 7.046 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.479 (BCF = 301.1)
Log BAF Arnot-Gobas method (upper trophic) = 2.482 (BAF = 303.1)
log Kow used: 3.50 (user entered)

Volatilization from Water:
Henry LC: 1.73E-008 atm-m3/mole (estimated by Bond SAR Method)
Half-Life from Model River: 6.327E+004 hours (2636 days)
Half-Life from Model Lake : 6.904E+005 hours (2.877E+004 days)

Removal In Wastewater Treatment:
Total removal: 13.03 percent
Total biodegradation: 0.18 percent
Total sludge adsorption: 12.85 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**0.025**	**155**	**1000**
Water	**3.12**	**4.32e+003**	**1000**
Soil	**83.9**	**8.64e+003**	**1000**
Sediment	**12.9**	**3.89e+004**	**0**

Persistence Time: 8.93e+003 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.025	155	1000
Water	3.12	4.32e+003	1000
water	(2.96)		
biota	(0.000468)		
suspended sediment	(0.154)		
Soil	83.9	8.64e+003	1000
Sediment	12.9	3.89e+004	0

Persistence Time: 8.93e+003 hr

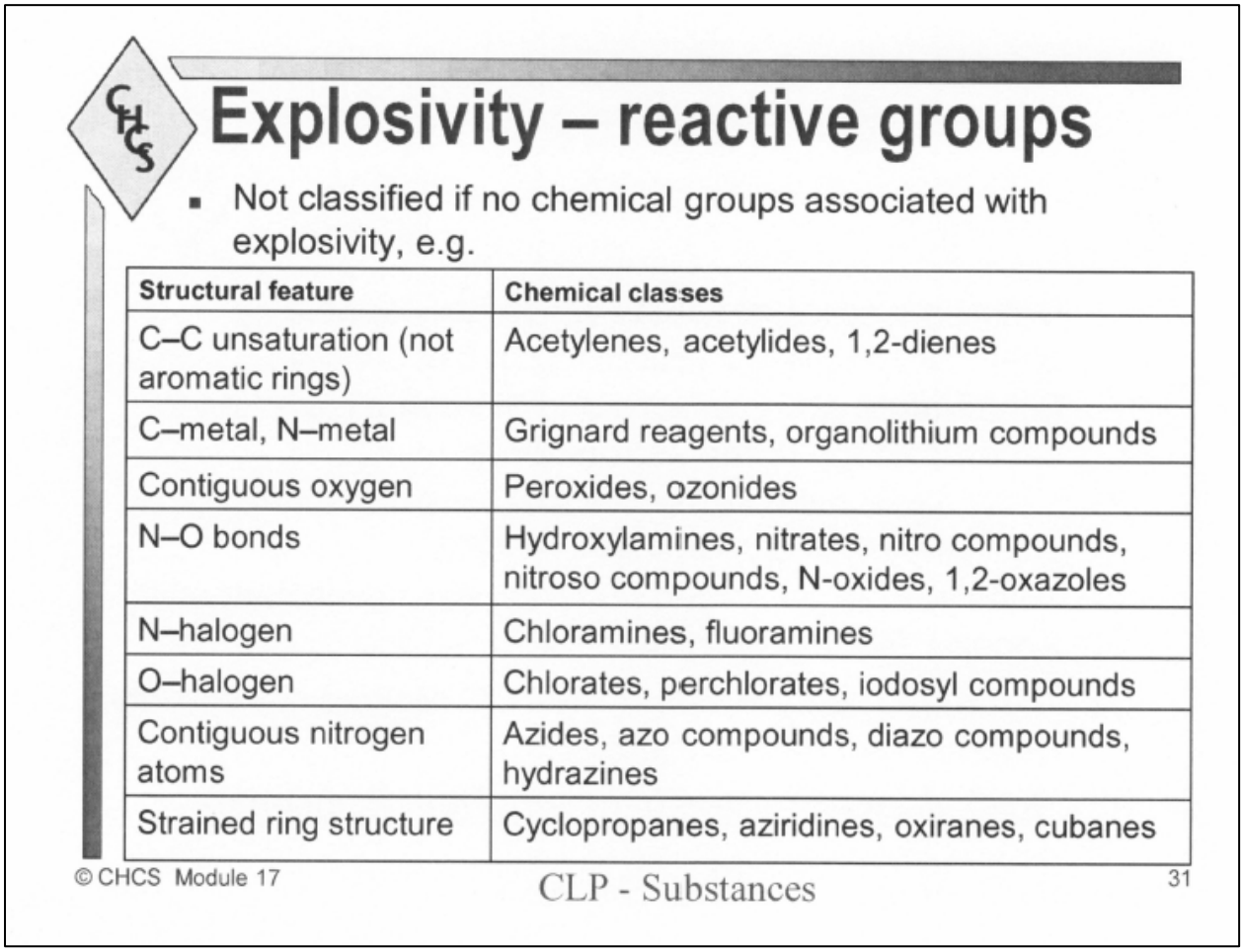
Level III Fugacity Model: (EQC Default)

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

Air 0.0311 155 1000
Water 4.97 4.32e+003 1000
water (4.96)
biota (0.000784)
suspended sediment (0.00964)
Soil 94.5 8.64e+003 1000
Sediment 0.541 3.89e+004 0
Persistence Time: 7.24e+003 hr

APPENDIX Q: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



Explosivity – reactive groups

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

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

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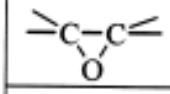
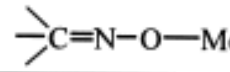
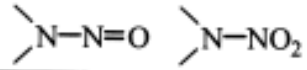
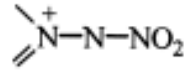
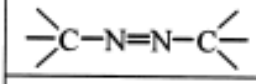
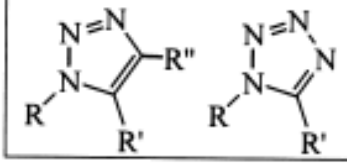
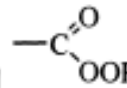
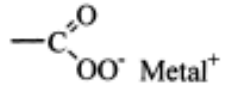
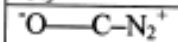
Explosivity – Full List

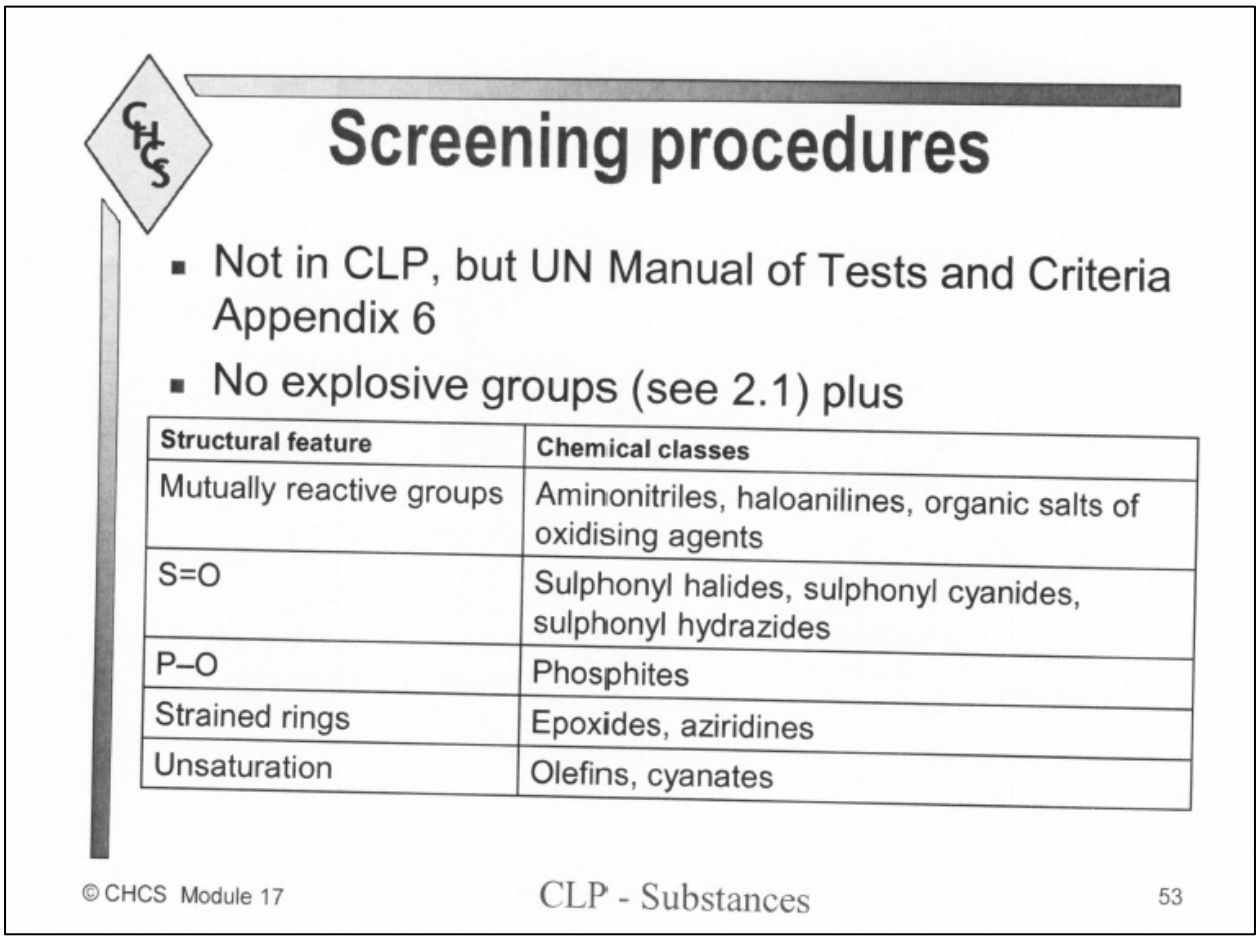
Table R.7.1-28 Chemical groups associated with explosive properties

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

Chemical group	Chemical Class
[1] ROOR', [2] 	Peroxy Compounds: [1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic); [2] Peroxo acids (R'=H), Peroxyesters (R'=organic)
[1] ROOMetal, [2] 	Metal peroxides, Peroxoacids salts
$-N_3$	Azides e.g. PbN_6, CH_3N_3
	Arenediazonium oxides i.e. inner diazonium salts in which the counter ion is an oxide
Ar-N=N-S- Ar-N=N-S-Ar	Diazonium sulfides and derivatives, Arenediazo Aryl Sulfides
XO_n	Halogen Oxide: e.g. percholrates, bromates, etc
NX_3 e.g. $NC1_3$, $RNC1_2$	N-Halogen Compounds

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

Self-Reactive Substances



APPENDIX R: Change in Benchmark Score

Table 6 provides a summary of changes to ToxServices' GreenScreen® Benchmark™ for tralopyril. The GreenScreen® Benchmark Score for tralopyril has changed over time. The original GreenScreen® assessment was performed in 2015 under version 1.2 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. In 2020, ToxServices changed the GreenScreen® benchmark score to a BM-1 due to reclassification of developmental endpoint from Low (low confidence) to High (low confidence), reclassification of single exposure neurotoxicity endpoint from Data Gap to Very High (low confidence), and reclassification of persistence endpoint from High (low confidence) to Very High (low confidence) following a weight of evidence evaluation of this chemical's updated dataset. The BM-1 score is maintained with this 2023 version 1.4 update.

Table 6: Change in GreenScreen® Benchmark™ for Tralopyril

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
January 4, 2015	BM-2	v. 1.2	
July 14, 2020	BM-1	v. 1.4	BM score changed to a BM-1 due to reclassification of developmental endpoint from *Low* (low confidence) to *High* (low confidence), reclassification of single exposure neurotoxicity endpoint from Data Gap to *Very High* (low confidence), and reclassification of persistence endpoint from *High* (low confidence) to *Very High* (low confidence).
August 29, 2023	BM-1	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template. The score for skin sensitization has changed from **L** (high confidence) to *L* (low confidence) based on re-evaluation of data and modeling. The scores for other endpoints remain the same.
October 16, 2023	BM-1	v. 1.4	No change in BM score. The GreenScreen® assessment was updated to correct typos, and clarify score justification based on comments from Washington Department of Ecology.

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