6-ETHOXY-2,2,4-TRIMETHYL-1,2-DIHYDROQUINOLINE (ETHOXYQUIN)

(CAS #91-53-2)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: November 8 2021

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GreenScreen® Executive Summary for 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) (CAS #91-53-2)

6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) is a quinoline organic compound with an ethoxy substituent that is used in animal feed as an antioxidant and is permitted as an anti-scald agent on apples and pears with a residue tolerance of 3 ppm (40 CFR § 180.178) and as a direct food additive (21 CFR § 172.140). The United States Food and Drug Administration (U.S. FDA) has established a tolerance of 0.5 ppm for residues of ethoxyquin in or on uncooked meat and meat by-product (including milk and eggs) and 5.0 ppm for cooked meat and meat by-products of animals fed forage crops or feed supplements treated with ethoxyquin. Ethoxyquin is used as a color preservative in spices and as an anti-degradation agent for rubber. Ethoxyquin is a yellow to brown liquid at standard temperature and pressure, not highly volatile, and is slightly soluble in water.

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

- Benchmark 2c
  - High Persistence-P + Moderate Group I Human Toxicity (reproductive toxicity-R, developmental toxicity-D and endocrine activity-E)
  - High P + Ver High Group II Human Toxicity (single dose systemic toxicity-STs)
  - High P + High Group II* Human Toxicity (repeated dose systemic toxicity-STr*)
  - High P + Moderate Group II Human Toxicity (acute toxicity-AT)
  - High P + Moderate Group II* Human Toxicity (skin sensitization-SnS*)
  - High P + High Ecotoxicity (acute aquatic-AA and chronic aquatic-CA)
- Benchmark 2e
  - Moderate Group I Human Toxicity (R, D and E)
- Benchmark 2f
  - High Group II* Human Toxicity (systemic toxicity-repeat dose-STr)
  - Very High Group II Human Toxicity (STs)

Data gaps (DG) exist neurotoxicity-single and repeat dose-Ns and Nr*, and respiratory sensitization-SnR*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), ethoxyquin meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if ethoxyquin were assigned a High score for the data gaps Nr* or SnR*, or a Very High score for Ns, it would still be categorized as a Benchmark 2 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for carcinogenicity, endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and *in vitro* assays for endocrine activity and genotoxicity. 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 ethoxyquin's NAMs dataset include insufficient *in vivo* experimental or human data for carcinogenicity, endocrine activity, respiratory sensitization, chronic aquatic toxicity and persistence, and lack of validated test methods for respiratory sensitization. Ethoxyquin's Type II (extrapolation output) uncertainties include uncertain *in vivo* relevance of *in silico* predictions and *in vitro* high throughput receptor binding assays of endocrine activity, the limitation of *in vitro* genotoxicity assays in mimicking metabolic systems, the lack of applicability domains for ToxCast

models for endocrine activity, the limitation of OECD Toolbox and Toxtree in identifying structural alerts without defining applicability domains, and OECD Toolbox not accounting for non-immunologic mechanisms of respiratory sensitization. Some of ethoxyquin's uncertainties can be alleviated by the use of *in vitro* and/or in combination with *in vivo* data, and ECHA's decision framework to evaluate respiratory sensitization.

GreenScreen® Hazard Summary Table for Ethoxyquin

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	**M**	**R**	**D**	**E**	**AT**	**ST**		**N**		**SnS**	**SnR**	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
						s	r*	s	r*	*	*								
L	**L**	*M*	*M*	**M**	**M**	**vH**	**H**	DG	DG	**M**	DG	**L**	**L**	**H**	*H*	*H*	**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 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) (CAS #91-53-2)

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

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Thea Clipson, Ph.D., M.S.
Title: Toxicologist
Organization: ToxServices LLC
Date: October 13, 2021, November 2, 2021

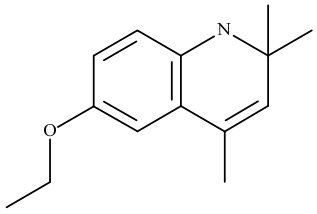
Quality Control Performed By:
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: October 15, 2021, November 8, 2021

Expiration Date: November 8, 2026[2]

Chemical Name: 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin)

CAS Number: 91-53-2

Chemical Structure(s):



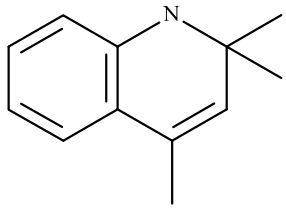
(ChemIDplus 2021a)

Also called:
Ethoxyquin [ISO]; 1,2-Dihydro-2,2,4-trimethyl-6-ethoxyquinoline; 1,2-Dihydro-6-ethoxy-2,2,4-trimethylquinoline; 2,2,4-Trimethyl-6-ethoxy-1,2-dihydroquinoline; 6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline; 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline; AI3-17715; Alterungsschutzmittel EC; Amea 100; Antage AW; Antioxidant EC; Aries Antox; Caswell No. 427D; CCRIS 2513; Dawe's nutrigard; EINECS 202-075-7; EMQ; EPA Pesticide Chemical Code 055501; EQ; Ethoxychin; Ethoxychin [Czech]; Ethoxyquine; HSDB 400; Niflex; Niflex D; Nix-Scald; Nocrac AW; Nocrack AW NSC-6795; Permanax 103; Quinol ED; Quinoline, 6-ethoxy-1,2-dihydro-2,2,4-trimethyl-; Santoflex A Santoflex AW; Santoquin; Santoquine; Santoquine (VAN); Stop-Scald; UNII-9T1410R4OR; USAF B-24 (ChemIDplus 2021a)

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
1,2-Dihydro-2,2,4-trimethylquinoline (DTMQ) (CAS 147-47-7) was identified as a surrogate for the carcinogenicity endpoint as sufficient data were not available for ethoxyquin and additionally, the United States Environmental Protection Agency (U.S. EPA) identified DTMQ as structurally similar to ethoxyquin in its pesticide reregistration assessment of ethoxyquin (U.S. EPA 2004). Due to uncertain

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

impact of the ethoxy side chain on the base structure in the target chemical, ToxServices considered DTMQ to be a weak surrogate.



Surrogate: DTMQ (CAS #147-47-7) (ChemIDplus 2021b)

Identify Applications/Functional Uses: (HSDB 2003)
1. Antioxidant
2. Pesticide/Insecticide
3. Antioxidant additive in animal feed
4. Antidegeneration agent for rubber
5. Medication
6. Color preservative in spices

Known Impurities[3]:
p-Phenetidine (CAS #156-43-4) is an impurity of the manufacturing process (EFSA 2015). The screen is performed on the theoretical pure substance.

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

- Benchmark 2c
  - High Persistence-P + Moderate Group I Human Toxicity (reproductive toxicity-R, developmental toxicity-D and endocrine activity-E)
  - High P + Ver High Group II Human Toxicity (single dose systemic toxicity-STs)
  - High P + High Group II* Human Toxicity (repeated dose systemic toxicity-STr*)
  - High P + Moderate Group II Human Toxicity (acute toxicity-AT)
  - High P + Moderate Group II* Human Toxicity (skin sensitization-SnS*)
  - High P + High Ecotoxicity (acute aquatic-AA and chronic aquatic-CA)
- Benchmark 2e
  - Moderate Group I Human Toxicity (R, D and E)
- Benchmark 2f
  - High Group II* Human Toxicity (systemic toxicity-repeat dose-STr)
  - Very High Group II Human Toxicity (STs)

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

Data gaps (DG) exist neurotoxicity-single and repeat dose-Ns and Nr*, and respiratory sensitization-SnR*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), ethoxyquin meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if ethoxyquin were assigned a High score for the data gaps Nr* or SnR*, or a Very High score for Ns, it would still be categorized as a Benchmark 2 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for Ethoxyquin

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

Ethoxyquin readily hydrolyzed to form seven degradation products, including methylated, demethylated, and deethylated quinoline and dimeric ethoxyquin through intermolecular rearrangement and dimerization at 25°C and pH of 5, 7 and 9 in the dark, with dissipation half-lives (DT50) of 3.7, 6.7 and 9.3 days at each pH, respectively. No additional details were provided for the identities of the degradants (Klimisch 2, reliable with restrictions) (ECHA 2021a). Representative structures deduced from the limited information provided are presented in Table 1, below. No data were identified for the biodegradation of ethoxyquin; therefore, ToxServices considered the hydrolysis and dimerization products relevant environmental transformation products. As these products are not LT-1 chemicals, the benchmark score of the parent compound is not modified.

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]
Any	Hydrolysis	Representative methylated ethoxyquin, SMILES: CCOc1ccc2N(C)C(C)(C)C=C(C)c2c1	N/A	Yes	Yes	Not in Pharos database
Any	Hydrolysis	Deethylated ethoxyquin, SMILES: CC1=CC(C)(C)Nc2ccc(O)cc12	N/A	Yes	Yes	Not in Pharos database

[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 2021) 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).

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]**
Any	Hydrolysis	Example of demethylated ethoxyquin, SMILES: CCOc1ccc2NC(C)(C)C=Cc2c1	N/A	Yes	Yes	Not in Pharos database
Any	Dimerization	Ethoxyquin dimer	74681-77-9	Yes	Yes	Not in Pharos database

Introduction

Ethoxyquin is a quinoline organic compound with an ethoxy substituent that is an approved food additive used in animal feed as an antioxidant, is permitted as an anti-scald agent on apples and pears with a residue tolerance of 3 ppm (40 CFR § 180.178) and as a direct food additive (21 CFR § 172.140). The U.S. FDA has established a tolerance of 0.5 ppm for residues of ethoxyquin in or on uncooked meat and meat by-product (including milk and eggs) and 5.0 ppm for cooked meat and meat by-products of animals fed forage crops or feed supplements treated with ethoxyquin. It is also used as a color preservative in spices and as an anti-degradation agent for rubber. Ethoxyquin is a yellow liquid which is slightly soluble in water and combustible (HSDB 2003).

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

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

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

Ethoxyquin is not listed on the SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2021) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[10] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for ethoxyquin can be found in Appendix C.

- Ethoxyquin is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Ethoxyquin is not listed on the U.S. DOT list.

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

- Ethoxyquin is on the following lists for multiple endpoints.
  - German FEA – Substances Hazardous to Waters – Class 2 – Hazard to Waters
  - Québec CSST – WHMIS 1988 – Class D2B – Toxic material causing other toxic effects
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

A European Union (EU) harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statement was identified for ethoxyquin, as indicated in Table 2. General personal protective equipment (PPE) recommendations are presented in Table 3, below. No occupational exposure limits (OELs) were identified.

Table 2: GHS H Statements for Ethoxyquin (CAS #91-53-2) (ECHA 2021b)

H Statement	H Statement Details
H302	Acute Tox. 4 – Harmful if swallowed

Table 3: Occupational Exposure Limits and Recommended Personal Protective Equipment for Ethoxyquin (CAS #91-53-2)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Use of suitable respiratory protective device in case of insufficient ventilation (Filter A), gloves (synthetic rubber – penetration time ≥ 8 h), safety glasses	ECHA 2021a	None identified	

Physicochemical Properties of Ethoxyquin

Ethoxyquin is a yellow to brown liquid at standard temperature and pressure that is slightly soluble in water. Its measured vapor pressure of 0.0346 Pa (0.000259 mmHg) indicates that it is not highly volatile, and it decomposes before boiling. The measured log K_{ow} of 3.39 at pH 7 suggests that it is not likely to bioaccumulate.

Table 4: Physical and Chemical Properties of Ethoxyquin (CAS #91-53-2)

Property	Value	Reference
Molecular formula	C14-H19-N-O	ChemIDplus 2021
SMILES Notation	CCOc1ccc2NC(C)(C)C=C(C)c2c1	ChemIDplus 2021
Molecular weight	217.3101	ChemIDplus 2021
Physical state	Liquid at 20°C and 1,013 hPa	ECHA 2021a
Appearance	Brown, slightly viscous	ECHA 2021a
Melting point	<-20°C (EU Method A.1 – solidification point)	ECHA 2021a
Boiling point	Decomposes before boiling at 150°C (US EPA PAG-63)	ECHA 2021a
Vapor pressure	0.0346 Pa at 25°C (EPA OPPTS 830.7950)	ECHA 2021a
Water solubility	101 mg/L at 20°C, pH 5 60 mg/L at 20°C, pH 7 70 mg/L at 20°C, pH 9 (EU Method A.6)	ECHA 2021a
Dissociation constant	4.56 at 22°C (OECD Guideline 112)	ECHA 2021a

Table 4: Physical and Chemical Properties of Ethoxyquin (CAS #91-53-2)		
Property	**Value**	**Reference**
Density/specific gravity	1.031 at 25°C (EPA OPPTS 830.7300)	ECHA 2021a
Partition coefficient	Log K_{ow} = 3.39, pH 7 Log K_{ow} = 3.18, pH 5 Log K_{ow} = 3.18, pH 9 Temperatures not specified (EU Method A.8)	ECHA 2021a

Toxicokinetics

- *Absorption*
  - Ethoxyquin was rapidly and significantly (>90%) absorbed via the oral route in rats (EC 2008a).
  - While no experimental data are available on dermal absorption, the molecular weight and log K_{ow} values suggest 100% dermal absorption as an appropriate default (EC 2008a).
- *Distribution*
  - Ethoxyquin is covalently bound to plasma proteins in rats after i.v. exposure. Rapid partitioning of ethoxyquin from blood into major tissues was observed in rats and mice exposed to 25 mg/kg by i.v. injection, with the highest concentrations found in the liver, kidney and adipose tissues in both rats and mice. There is some evidence of redistribution from a deep compartment such as adipose tissue back to blood based on a second increase of blood levels from 12 – 24 hours after dosing.
- *Metabolism*
  - O-deethylation and conjugation to endogenous substrates are the two major metabolism pathways (Sanders et al. 1996, Burka et al. 1996). Following intragastric administration of ^{14}C-labeled ethoxyquin to bile duct cannulated rats, averages of 28 and 36% of the dose of radioactivity was recovered in the bile 12 and 24 hours, respectively (HSDB 2003). Radioactive substances recovered in the bile included ethoxyquin, 8-hydroxyethoxyquin, hydroxylated 8-hydroxyethoxyquin, 6-ethoxy-2,2,4-trimethylquinolone, hydroxylated 6-ethoxy-2,2,4-trimethyl-8-quinolone, 6-ethoxy-2,4-dimethylquinoline and 2,2,4-trimethyl-6-quinolone.
  - After i.v. exposure, in the rat, sulphate conjugates excreted in the urine included 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline sulphate, and 1,2,3,4-tetrahydro-3,6-dihydroxy-4-methylene-2,2-dimethylquinoline sulphate. In the mouse, a glucuronide, 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline glucuronide and one primary sulphate conjugate, 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline sulphate were detected in the urine (Sanders et al. 1996, Burka et al. 1996).
- *Excretion*
  - Ethoxyquin is rapidly and almost completely (90%) excreted in the urine and feces of rats and mice after oral or i.v. administration of single doses up to 25 mg/kg (Sanders et al. 1996, Burka et al. 1996).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

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

Ethoxyquin was assigned a score of Low for carcinogenicity based on limited but negative experimental

data in rats and dogs by the oral route. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate data are available and negative (CPA 2018b). The confidence in the score is low as none of the studies identified were conducted according to modern guidelines, the weak surrogate DTMQ produced some evidence of carcinogenicity in rats, and modelled data are mixed.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- NTP 1990
  - The U.S. FDA nominated ethoxyquin for carcinogenicity testing in 1990 based on the increased use and high levels of ethoxyquin in animal feed (150 ppm). It is also structurally similar to Quindoxin, a known carcinogen, and Flectol-H, a tumor inducer in rats. To date, carcinogenicity testing has not been carried out.
- EC 2008b
  - *Oral:* In a non-guideline 2-year study, groups of rats (10/sex, strain not specified) were exposed to ethoxyquin in the diet at 0, 62, 125, 250, 500, 1,000, 2,000 or 4,000 ppm. Necropsy was performed after 200, 400, 600 and 715 days. There were no treatment related effects on survival. Significantly reduced body weight gain was measured only occasionally without dose response. No tumors were found. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) noted that the number of animals in this study was too few to detect rare tumors, although the addition of interim sacrifices and the wide dose range alleviated the concerns.
  - *Oral:* In a non-guideline 18-month study designed to investigate the progression of renal lesions, F344 rats (6/19/sex/dose) were exposed to ethoxyquin in the diet at 0 or 5,000 ppm, and another group was exposed to 5,000 ppm (equivalent to 250 mg/kg/day according to EC report) for 24 weeks followed by 34 weeks of control diet. Interim sacrifices were performed at 4, 12, 14, 24, 58 and 78 weeks. There was no evidence of preneoplastic proliferative lesions.
  - *Oral:* A non-guideline 30-week oral study was carried out to study the age and sex dependence of renal lesions produced by ethoxyquin. F344 rats were exposed in the diet at 5,000 ppm (250 mg/kg/day according to EC report). The histopathological examination focused on the kidney and did not seem to identify any neoplastic lesions.
  - *Oral:* A non-guideline 5-year study was performed in Beagle dogs (14 male and females/group) exposed to 0 and 3,000 ppm (equivalent to 7.5 mg/kg/day according to JMPR). There were no treatment related effects on hematology, urinalysis, clinical chemistry, organ weights, body weight, gross pathology, or histopathology.
  - *Intracutaneous:* Neonatal Swiss ICR/Ha mice (28 – 57 per dose) were exposed to ethoxyquin solutions by intracutaneous injection at concentrations of 10 or 50 mg/mL on postnatal days (PNDs) 1 and 7 (0.1 mL), and 14 and 21 (0.2 mL), or as a single dose of 100 mg/mL (0.1 mL) on PND 1. The doses on PND 1 were equivalent to 500, 2,500 and 5,000 mg/kg and 250 and 1,250 mg/kg on PND 21, according to the EC report. Animals were terminated at week 53. Limited tissues and lesions underwent tumor examinations. Study authors concluded that four intracutaneous exposures of ethoxyquin at near-lethal doses to neonatal mice were not carcinogenic at 1 year of age.
  - EC report authors concluded that ethoxyquin was not carcinogenic in rats and dogs, and the study in mice was too limited to arrive at a meaningful conclusion.
- NTP 1997
  - *Dermal*: *Surrogate: DTMQ (CAS# 147-47-7):* In 2-year carcinogenicity studies conducted by the National Toxicology Program (NTP), groups of 60 male and 60 female F344/N rats

or B6C3F1 mice were topically administered 0, 36, 60, or 100 mg/kg/day and 0, 3.6, 6, or 10 mg/kg/day DTMQ (> 90% purity) in acetone, respectively, 5 days per week for 103 (males) or 104 (females) weeks. Nine or ten animals per group were evaluated after 15 months of treatment. In rats, treatment caused several non-neoplastic skin lesions such as in males and females at the mid and high dose groups such acanthosis in males and females and hyperkeratosis in females at the site of application. In addition, absolute and relative right kidney weights of 60 and 100 mg/kg male rats were significantly greater than those of the controls at the 15-month interim evaluation. Incidences of renal tubule adenoma and adenoma or carcinoma (combined) in all treated groups of males were significantly/greater than those in the controls. These incidences exceeded the range from the historical controls in 2-year NTP feed studies. An extended (step section) evaluation of the kidneys of male rats did not reveal an additional increase in neoplastic response because additional adenomas and hyperplasias were observed in the controls as well as in treated groups. Based on increased incidences of renal tubule adenoma and adenoma or carcinoma (combined)[11], the NTP concluded that there was some evidence of carcinogenic activity of DTMQ in male F344/N rats but not in females. In mice, there was no evidence of carcinogenic activity and no nonneoplastic lesions were observed in male or female mice attributed to treatment with DTMQ.

- DTU 2021 (Appendix D)
    - QSAR modeling with the Danish (Q)SAR database resulted in the following predictions for ethoxyquin:
        - The Leadscope model predicted the compound to be negative in all of the FDA RCA Cancer models (7/7). The compound was within the applicability domain for 6 of the 7 models.
        - The E Ultra model predicted the compound to be negative for carcinogenicity with all of the FDA RCA Cancer models (7/7) and the compound was within the applicability domain for all 7.
        - For liver specific cancer in the rat or mouse, results were as follows:
            - Battery – inconclusive, and out of the applicability domain
            - CASE Ultra – inconclusive, and out of the applicability domain
            - Leadscope – negative, but out of the applicability domain
            - SciQSAR – negative, but out of the applicability domain
- Toxtree 2018
    - Toxtree predicts ethoxyquin will not be a nongenotoxic or genotoxic carcinogen using the rulebase by ISS (Appendix E).
- VEGA 2021
    - Ethoxyquin was determined to be a non-carcinogen by the Carcinogenicity model (CAESAR) 2.1.9 with high reliability based on experimental data and modeling (Global applicability domain (AD) Index = 1, similarity index = 1, accuracy index = 1, concordance index = 1) (Appendix F).
    - Ethoxyquin was predicted to be a non-carcinogen by the Carcinogenicity model (ISS) 1.0.2 with low reliability (Global AD Index = 0, similarity index = 0.868, accuracy index = 0.495, concordance index = 0) (Appendix F).

[11] NTP interprets some evidence of carcinogenicity as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

  - Ethoxyquin was predicted to be a non-carcinogen by the Carcinogenicity model (IRFMN/Antares) 1.0.0 with low reliability (Global AD Index = 0, similarity index = 1, accuracy index = 0, concordance index = 0) (Appendix F).
  - Ethoxyquin was predicted to be a carcinogen by the Carcinogenicity model (IRFMN/ISSCAN-CGX) 1.0.0 with high reliability (Global AD Index = 0.917, similarity index = 0.842, accuracy index = 1, concordance index = 1) (Appendix F).
  - Ethoxyquin was predicted to be a carcinogen by the oral Carcinogenicity model (IRFMN) 1.0.0 with moderate reliability (Global AD Index = 0.752, similarity index = 0.797, accuracy index = 0.504, concordance index = 1) (Appendix F).
  - Ethoxyquin was predicted to be a carcinogen by the inhalation Carcinogenicity model (IRFMN) 1.0.0 with moderate reliability (Global AD Index = 0.752, similarity index = 0.797, accuracy index = 0.504, concordance index = 1) (Appendix F).
- U.S. EPA 2021b
  - An attempt was made to evaluate the carcinogenic potential of ethoxyquin using OncoLogic™; however, this class of chemicals could not be evaluated in the current version.
- Based on the weight of evidence, a score of Low was assigned. Limited experimental data on ethoxyquin indicate a lack of carcinogenic potential by the oral route, as concluded by the EC report authors. Toxtree predicted that ethoxyquin will not be a nongenotoxic or genotoxic carcinogen. VEGA models produced mixed results. Three of the six models predicted ethoxyquin to be a non-carcinogen with the global AD index >0.7 in one of the three models, indicating a reliable prediction. However, three of the six models predicted it to be a carcinogen, and the reliability was acceptable (global AD index >0.7) in all three models. Danish (Q)SAR Database models predicted ethoxyquin will not be a carcinogen in all of the models that produced in domain predictions. The OncoLogic software was unable to evaluate the carcinogenic potential of ethoxyquin. ToxServices relied on experimental data on the target chemical to score this endpoint but adjusted the confidence level based on the mixed predictions from VEGA models with high reliability, and some evidence of carcinogenic potential of the weak surrogate.

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

Ethoxyquin was assigned a score of Low for mutagenicity/genotoxicity based on negative results for clastogenicity and DNA repair in *in vivo* assays, although there are positive results for mutagenicity and clastogenicity obtained in *in vitro* assays. 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 as it is based on reliable *in vivo* experimental data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - *In vitro:* In a GLP-compliant bacterial reverse mutation assay, conducted in accordance with OCED Guideline 471, *Salmonella typhimurium* test strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP_2 *uvrA* were tested at concentrations of 10, 33.3, 100, 333, 1,000, 2,000 µg/plate ethoxyquin (purity not specified; vehicle solvent not specified) in *S. typhimurium* test strains and at concentrations 33.3, 100, 333, 1,000, 3,330, and 5,000 µg/plate ethoxyquin (purity not specified; vehicle solvent not specified), with and without metabolic activation. S9 metabolic activation mix was derived from rat liver induced with Aroclor. Negative solvent/vehicle controls and positive controls were used but not specified. No mutagenic activity was observed under the conditions of this study (Klimisch

score 2 – reliable with restrictions).

- *In vitro:* In a GLP-compliant mammalian chromosome aberration test, conducted in accordance with OECD Guideline 473, Chinese hamster ovary (CHO) cells were tested at concentrations of 1.25, 2.50, 5.00, 10.0; 15.0, 20.0, 30.0, 50.0, 75.0 and 100 µg/mL without metabolic activation and 5.00, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0 and 50.0 µg/mL ethoxyquin (purity not specified) in DMSO with metabolic activation. Metabolic activation was identified as an exogenous metabolic activation system. Positive controls with and without metabolic activation were cyclophosphamide and mitomycin C. Under the conditions of the study, ethoxyquin induced a significant increase in cells with chromosomal aberrations in cultures treated with 20.0 and 30.0 µg/mL without metabolic activation as well as a significant increase in polyploidy in cultures treated with 30.0 µg/mL, but no evidence of endoreduplication. With metabolic activation, there was a significant increase in cells with chromosomal aberrations at concentrations of 15.0, 20.0, and 25.0 µg/mL and a significant increase in polyploidy at 20.0 µg/mL, along with a significant increase in endoreduplication at concentrations of 10.0, 15.0, and 20.0 µg/mL. Under the conditions of the study, ethoxyquin induced chromosomal aberrations above background (Klimisch score 2 – reliable with restrictions)**.**
- *In vitro:* In a GLP-compliant mammalian cell gene mutation assay conducted according to OECD Guideline 476, mouse lymphoma L5178Y cells were tested at concentrations of 5-25 µg/mL and 1.3-4.4 µg/mL ethoxyquin (purity not reported) in DMSO, with and without metabolic activation, respectively. S9 metabolic activation mix was derived from rat liver induced with Aroclor 1254. Positive controls were not specified. Ethoxyquin was moderately to highly toxic, with a concentration of 15 µg/mL reducing the relative suspension growth (RSG) to 49.1% after a 4-hour exposure. Ethoxyquin was more toxic in the presence of metabolic activation, with the RSG reduced to 55% and 0.5% at 1.5 and 5 µg/mL, respectively. Ethoxyquin induced gene mutations in mouse lymphoma L5178Y cells with and without metabolic activation (Klimisch score 2 – reliable with restrictions).
- *In vivo:* In a GLP-compliant mammalian erythrocyte micronucleus test conducted according to OECD Guideline 474, male Crl:CD-1®(ICR)BR mice (6/dose) were administered a single oral gavage dose of 0, 375, 750, or 1,500 mg/kg/day ethoxyquin (purity not specified) in corn oil. The positive control was 80 mg/kg cyclophosphamide. Animals were sacrificed 24 or 48 hours after treatment and bone marrow was extracted. Bone marrow was examined for polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs). Three animals in the 1,500 mg/kg treatment group died and there were signs of clinical toxicity in animals in the 750 and 1,500 mg/kg treatment groups. Clinical signs of toxicity included hypoactivity, squinted eyes, irregular respiration, ataxia, and temporary trace (standing on hind limbs with upright head with reduced movement). Ethoxyquin did not induce a statistically significant increase in micronucleated PCEs at any of the dose levels and it was not cytotoxic to the bone marrow with no significant effect on the PCE/NCE ratios (Klimisch score 2 – reliable with restrictions).
- *In vivo:* In a GLP-compliant unscheduled DNA (UDS) test with mammalian liver cells conducted according to OECD Guideline 486, male Sprague-Dawley rats (5/dose) were administered two oral gavage doses of 0, 225, or 750 mg/kg ethoxyquin (purity not specified) in corn oil, with the second dose given 14 hours after the first and 2 hours before sacrifice and liver perfusion. The positive control was one oral dose of 75 mg/kg 2-acetylaminofluorene (2-AAF) and 16 hours before liver perfusion. Hepatocytes were isolated by enzymatic dissociation and examined *ex vivo* for UDS. All animals survived until sacrifice at 2 hours after the second dose. Signs of toxicity were observed in the high dose group after the second dose, including reduced activity, irregular respiration, unsteady

gait or reduced body tone. Ethoxyquin did not induce unscheduled DNA repair in the hepatocytes of treated rats (Klimisch score 2 – reliable with restrictions).

- NTP 1991
  - *In vitro:* Ethoxyquin was not clastogenic in a chromosome aberration test in which CHO cells were exposed to concentrations of 25, 54, 116, or 250 µg/mL ethoxyquin with and without metabolic activation. Metabolic activation was derived from induced rat liver S9. Cyclophosphamide was used as positive control at a concentration of 20 µg/mL.
  - *In vitro:* Ethoxyquin was mutagenic in a sister chromatid exchange test in which CHO cells were exposed to concentrations between 1.7 and 500 µg/mL ethoxyquin without metabolic S9 activation but negative for mutagenicity with metabolic activation at concentrations between 17 and 500 µg/mL. Cyclophosphamide was used as a positive control at concentrations of 0.125 and 0.5 µg/mL.
- NTP 1998a
  - *In vitro:* Ethoxyquin was not mutagenic in an Ames assay using *S. typhimurium* test strains TA100, TA1535, TA97, TA98, and TA104 with and without 10% and 30% rat S9, and 10 and 30% hamster S9 at concentrations ranging between 0.3 and 1,000 µg/plate. Positive controls were 2-aminoanthracene (with metabolic activation), sodium azide (TA1535 and TA100 without metabolic activation), 9-aminoacridine (TA97 without metabolic activation), and 4-nitro-O-phenylenediamine (TA98 without metabolic activation).
- NTP 1998b
  - *In vivo:* Ethoxyquin was not clastogenic in an *in vivo* micronucleus assay in which B6C3F1 mice (5/dose, except 3/highest dose) received 3 intraperitoneal injections of 400, 600, 800, or 1,000 mg/kg ethoxyquin. Animals were sacrificed 24 hours after final treatment, and bone marrow were extracted. Cyclophosphamide was used as a positive control at a concentration of 25.0 mg/kg. Ethoxyquin did not induce micronucleated PCEs at any of the dose levels.
- While positive results were obtained in an *in vitro* chromosome aberration assay and an *in vitro* mouse lymphoma assay, EU attributed the observation of ethoxyquin-induced forward mutations in the mouse lymphoma cell mutation assay to chromosomal damage rather than point mutations after mutant colonies assessment (EC 2008a). Therefore, *in vitro* data indicate a potential for chromosomal damage. However, *in vivo* micronucleus assays were negative, indicate a lack of clastogenicity *in vivo.*

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

Ethoxyquin was assigned a score of Moderate for reproductive toxicity based on decreased number of litters per pair and increased gestation length in a 2-generation reproductive toxicity study in rats at a LOAEL of 300 mg/kg/day, in the presence of maternal systemic toxicity. No effects on reproduction in a two-generation reproductive toxicity study in dogs up to the highest dose tested, 5.6 mg/kg/day. GreenScreen® criteria classify chemicals as a Moderate hazard for reproductive toxicity when there is limited or marginal evidence of reproductive toxicity (CPA 2018b). The confidence in the score is low as it is based on a study with limited reporting and the effects may be secondary to maternal toxicity.

- 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 2021a
  - In a non-GLP, non-guideline (peer-reviewed) two-generation reproductive toxicity study that included developmental endpoints, male and female beagle dogs (F0: 5 males and 10 females/treatment group; F1: 8 males and 13 female pups/treatment group) were fed diets

containing 0, 100, or 225 ppm ethoxyquin (purity not specified) (approximately 2.5 and 5.6 mg/kg/day, respectively) for a minimum of 82 days before pairing. For the F1 matings, 8 male and 13 female pups were fed diets containing 0, 100, or 225 ppm ethoxyquin from weaning until breeding at an age of 10-30 months (and the 2nd estrus cycle in females). Routine physical examinations were performed, and animals were regularly observed. Semen samples were collected during the first week of treatment and at the time of mating in order to record volume, sperm count, motility, velocity, and morphology. Urine and blood samples were collected for hematology, clinical chemistry, and urinalysis from fasted adults before treatment and at the end of the F0 phase, at weeks 10, 23, 36, 49 and 62, and at the end of the F1 growth phase, and at the conclusion of the F1 mating phase. Ophthalmological examinations were carried out at the beginning and end of the F1 growth and matin phases. Mating, birth (whelping), and lactation indices were recorded. F1 adults and pups showing signs of toxicity were necropsied and tissues (not specified) from controls and F1 animals were evaluated histologically, as well as tissues (not specified) from F2 pups showing signs of toxicity. Livers and gall bladders of F1 adults at the low dose and adrenal glands and spleens from F1 adult females at the low dose were analyzed. Macroscopic and microscopic evaluations were carried out on F0 and F1 animals that died or were sacrificed due to clinical signs of toxicity. A statistically significant increase in serum alkaline phosphatase activity was measured in F0 males in the high dose group and in F0 females in the low and high dose groups. Two pregnant females in the high dose group did not give birth. There were no differences between treated and control animals in terms of mating performance, labor, birth, or weaning indices, semen parameters, or clinical signs, as well as no differences in litter size, pup survival, and pup weight and growth. A NOAEL ≥ 5.6 mg/kg/day was estimated for reproductive performance for the first parental generation (P0). In the second parental generation (P1), at 225 ppm (approximately 5.6 mg/kg/day), there was an increase in the number of both male and female pups with a raw or red anus, dehydration, nasal discharge, excessive lachrymation, thinness, and pale gums. Nasal discharge and excessive lachrymation were also observed in the low dose group. Treatment related reductions in erythrocyte count, hematocrit, and hemoglobin were observed, and partial thromboplastin time were reduced in treated females. Serum activities of alkaline phosphatase, gamma-glutamyl transferase (GT), and alanine aminotransferase were increased in animals in the high dose group, indicating impaired liver function. In the F1 high dose mating group, statistically significant reductions in glucose, cholesterol, protein, albumin, and albumin:globulin ratio were observed, along with increases in total bilirubin concentration and in gamma-GT, alkaline phosphatase, and alanine aminotransferase activities. Increases in the absolute weights of the spleen and testes and both organs relative to brain weight were observed in treated males. Lesions in the liver of one high dose male and in the cervical lymph nodes of two high dose females were noted. Pigment protoporphyrin IX was observed in livers of 7/13 females at the low dose, 2/7 males at the high dose, and 10/11 females at the high dose. Fibrosis and hemorrhage frequencies were increased in the spleens of females in the high dose and there was also an increase in the incidence of pituitary cysts in animals in the high dose group. There were no treatment-related effects on reproductive performance, in terms of semen analyses or mating, gestation, whelping, or weaning indices; therefore, a NOAEL ≥ 5.6 mg/kg/day was estimated for reproductive performance for P1 animals. In the F1 generation, signs of clinical toxicity were observed in treated animals, including raw or red anus, dehydration, nasal discharge, and excessive lachrymation. A systemic toxicity LOAEL of 2.5 mg/kg/day was assigned due to clinical signs of parental toxicity, including excessive lachrymation and dehydration, and changes in clinical chemistry and pigment deposition in the liver of treated animals at

the low dose in both the F0 and F1 generations (Klimisch score 2 – reliable with restrictions).

- EC 2008b
    - In a two-generation study with limited reporting from an abstract, ethoxyquin was tested at 0, 75, 150 or 300 mg/kg/day in by gavage in rats (strain unspecified, 20/sex/group) following a continuous breeding protocol. Animals were individually housed for one week followed by cohabitation in breeding pairs for 16 weeks. Litters were terminated after evaluation on PND 1 during this period. Litters born after week 17 were reared until weaning on PND 21. Selected weanlings (F1) were reared to PND 81, then mated within groups to produce F2 generation, and sacrificed after delivery of the second generation litters. Parental systemic toxicity LOAEL was established by study authors at the lowest dose, 75 mg/kg/day, based on kidney and liver effects (increased weights by 13-56%). Study authors identified a reproductive toxicity NOAEL of 150 mg/kg/day and LOAEL of 300 mg/kg/day based on decreased number of litters per pair (by 22%), decreased number of live pups per litter (by 22%), increased gestation length and decreased live pup weight for the F0 generation evaluation. Crossover mating indicated that these effects were female-mediated. Study authors identified a developmental toxicity LOAEL of 75 mg/kg/day without statement of basis. No additional details were available.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *M*
Ethoxyquin was assigned a score of Moderate for developmental toxicity based on reduced pup body weight in the presence of maternal toxicity in a 2-generation reproductive toxicity study in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for developmental toxicity when there is limited or marginal evidence of developmental toxicity (CPA 2018b). The confidence in the score is low as it is based on a study with limited reporting, and the effects may be secondary to maternal systemic toxicity.

- 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 2021a
    - In the previously described, non-GLP, non-guideline (peer-reviewed) two-generation reproductive toxicity study that included developmental endpoints, male and female beagle dogs (F0: 5 males and 10 females/treatment group; F1: 8 males and 13 female pups/treatment group) were fed diets containing 0, 100, or 225 ppm ethoxyquin (purity not specified) (approximately 2.5 and 5.6 mg/kg/day, respectively) for a minimum of 82 days before pairing. For the F1 matings, 8 male and 13 female pups were fed diets containing 0, 100, or 225 ppm ethoxyquin from weaning until breeding at an age of 10-30 months (and the 2nd estrus cycle in females). Routine physical examinations were performed, and animals were regularly observed. Semen samples were collected during the first week of treatment and at the time of mating in order to record volume, sperm count, motility, velocity, and morphology. There were no increases in treatment-related malformations or variations in the pups whose parents were exposed to ethoxyquin. There were a few pups in the treated groups that showed nervous system dysfunction but this observation was attributed to the inheritance of an autosomal recessive trait in the breeding colony and not related to ethoxyquin treatment. According to the REACH dossier, the developmental endpoint is covered by this study and therefore, no additional studies are required and a NOAEL of 225 ppm (approximately 5.6 mg/kg/day) can be assigned for the developmental toxicity endpoint (Klimisch score 2 – reliable with restrictions).

- EC 2008b
    - In the previously described two-generation study with limited reporting from an abstract, ethoxyquin was tested at 0, 75, 150 or 300 mg/kg/day in by gavage in rats (strain unspecified, 20/sex/group) following a continuous breeding protocol. Animals were individually housed for one week followed by cohabitation in breeding pairs for 16 weeks. Litters were terminated after evaluation on PND 1 during this period. Litters born after week 17 were reared until weaning on PND 21. Selected weanlings (F1) were reared to PND 81, then mated within groups to produce F2 generation, and sacrificed after delivery of the second generation litters. Parental systemic toxicity LOAEL was established by study authors at the lowest dose, 75 mg/kg/day, based on kidney and liver effects (increased weights by 13-56%). Study authors identified a reproductive toxicity NOAEL of 150 mg/kg/day and LOAEL of 300 mg/kg/day based on decreased number of litters per pair (by 22%), decreased number of live pups per litter (by 22%), increased gestation length and decreased live pup weight for the F0 generation evaluation. Crossover mating indicated that these effects were female-mediated. Study authors identified a developmental toxicity LOAEL of 75 mg/kg/day without statement of basis. No additional details were available.
- EC 2008c
    - In a GLP-compliant prenatal developmental toxicity study conducted according to U.S. EPA FIFRA guideline for rodent teratogenicity, pregnant Sprague-Dawley rats (25/dose) received ethoxyquin by daily gavage at 0, 50, 150 or 350 mg/kg/day on gestation days (GDs) 6 – 19, and sacrificed on GD 20. Study authors identified a NOAEL of 50 mg/kg/day and LOAEL of 150 mg/kg/day for maternal toxicity based on clinical observations (staining in the urogenital area and various body surfaces, dark-colored urine), and reduced body weight gain and food consumption. Study authors identified a NOAEL of 350 mg/kg/day for developmental toxicity based on the lack of developmental effects observed.

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

Ethoxyquin was assigned a score of Moderate for endocrine activity based on antiandrogenic effects exhibited in an *in vitro* screening study of 200 pesticides, and some positive high throughput *in vitro* screening assays for estrogen receptor, androgen receptor, steroidogenesis, and thyroid receptor activities. However, no relevant health effects mediated through endocrine pathway were identified. In addition, altered thyroid hormone levels and enlarged thyroids were found in a subchronic oral study in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for endocrine activity when there is evidence of endocrine activity and when they are listed on TEDX for endocrine disruption. The preliminary Moderate score is raised to a High when there are linked adverse health effects warranting High scores for other relevant endpoints (CPA 2018b). Confidence in the score is high as it is based on changed thyroid hormone levels measured *in vivo*.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* TEDX – Potential Endocrine Disruptors
        - Kojima et al. 2004
            - Ethoxyquin exhibited antiandrogenic effects on androgenic activity (hAR – human androgen receptor) induced by dihydrotestosterone (DHT) in CHO cells.
- U.S. EPA 2021c
    - Ethoxyquin was active in 4/19 estrogen receptor (ER) assays, 3/14 androgen receptor (AR) assays, 7/26 steroidogenesis assays, and 6/15 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century.

  - Ethoxyquin was predicted to be inactive for androgen receptor agonism, antagonism, and binding using the COMPARA (consensus) model in ToxCast.
- DTU 2021
  - Modeling in the Danish (Q)SAR database provides the following results that are within the applicability domains of the models (see Appendix H).
    - Ethoxyquin is predicted to be negative for estrogen receptor activation (CERAPP data *in vitro*) by the Leadscope model.
    - 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) is predicted to be negative for androgen receptor activation (CoMPARA data *in vitro*) by the Leadscope model.
- ECHA 2021a
  - *Oral:* Ethoxyquin was evaluated in a repeated dose subchronic oral toxicity study conducted according to EPA OPP 82-1 (GLP-compliant. Male and female Sprague-Dawley rats (10/sex/dose) were exposed to the test substance (purity not specified) by gavage in corn oil, once daily for 90 days at 0, 20, 40, 200, and 400 mg/kg/day. Clinical chemistry changes were observed in the 200 and 400 mg/kg/day dose animals, including increased thyroid stimulating hormone (TSH) (in 200 mg/kg/day dose males, only). Mean thyroxine was reduced in the high dose males. Mean liver and kidney weights (absolute and relative to final body weight) were increased in the 200 and 400 mg/kg/day dose groups, and reddened and/or enlarged thyroid glands in the 200 and 400 mg/kg/day dose groups were observed at the 400 mg/kg/day dose (Klimisch score 2 – reliable with restrictions).

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

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

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

Ethoxyquin was assigned a score of Moderate for acute toxicity based on GHS Harmonized EU classification of Category 4 for the oral exposure route and a reported oral LD_{50} value between 300 and 2,000 mg/kg, an inhalation LC_{50} value between 1 and 5 mg/L, and a dermal LD_{50} value between 1,000 and 2,000 mg/kg, which correspond to GHS Category 4 classification. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when data for the most sensitive route of exposure meets the criteria for GHS Category 4 classification (CPA 2018b). The confidence in the score is high as it is based on a Harmonized EU classification of Category 4 and experimental data for three routes of exposure.

- Authoritative and Screening Lists
  - *Authoritative:*
    - EU – GHS – Acute Toxicity (oral) – Category 4 [H302]
  - *Screening:*
    - GHS – Australia – Acute Toxicity (oral) – Category 4 [H302]
    - GHS – Japan – Acute Toxicity (oral) – Category 4 [H302]
      -
    - GHS – New Zealand – 6.1D (oral) – Acutely Toxic [H302]
      - Oral LD_{50} = 800 mg/kg in rats; Dermal (24 hr) LD_{50} > 2,000 mg/kg in rats (NZ EPA 2021).
- ChemIDplus 2021
  - *Oral*: LD_{50} (mouse) = 1,584 mg/kg

  - *Oral*: LD_{50} (rat) = 800 mg/kg
- ECHA 2021a
  - *Oral*: Ethoxyquin (purity not specified) was evaluated for acute oral toxicity in a GLP-compliant study conducted in a manner similar to OECD Guideline 401. Albino rats (Crl:CD®BR) (5/sex/dose) were orally exposed by gavage to the test substance at 1,500, 1,950, and 2,535 mg/kg, followed by a 14-day observation period. Deaths of treated animals occurred withing 3 days of dosing, with the following mortalities: 2/10, 9/10, and 8/10 for the 1,500, 1,950, and 2,535 mg/kg dose groups, respectively. Clinical indicators of toxicity included ataxia in 24 animals, hypoactivity (21 animals), ocular discharge (16 animals), urogenital staining, hypothermia (cool to the touch, 13 animals), dried red material around the eyes, forelimbs, and nose, labored breathing (11 animals), prostrate positioning (10 rats), abnormal excretion (5 rats), and dried yellow material around the mouth. Surviving animals appeared normal, aside from the observation of dried yellow material around the mouth. There was no treatment-related effect on body weight. Histopathological examination revealed irritation in the gastrointestinal tract, and three animals exhibited hemorrhagic thymus glands. Dark red lungs and red fluid in the urinary bladder were observed in two rats. Enlarged cervical lymph nodes were noted in one animal and eye opacity in another animal. No other gross necropsy findings were observed in the animals that survived to planned euthanization. An oral LD_{50}s of 1,779, 1,675, and 1,726 mg/kg were calculated for males, females, and combined, respectively (Klimisch score 2 – reliable with restrictions)
  - *Inhalation*: Ethoxyquin was evaluated for acute inhalation toxicity in a GLP-compliant study conducted in a manner similar to OECD Guideline 403. Crl:CD®BR rats (5/sex/dose) were exposed whole body to an aerosol of 1.97 mg/L ethoxyquin (purity not specified) (100 % of the particles < 10 microns and 13.4 % < 1.18 microns) for 4 hours, with a 14-day post administration observation period. One animal was sacrificed on Day 1 for humane reasons, while all other animals survived the dosing and observation periods. Clinical observations included salivation during dosing, wet and/or dried yellow material on external body surfaces, dried red material around the nose, and dried yellow material around the eyes. There were no other significant clinical observations or effects on body weight during the study; however, the study authors suggest that tremors in 6/10 animals following treatment might indicate neurotoxic potential of ethoxyquin. A LC_{50} > 1.97 mg/L was assigned (Klimisch score 2 – reliable with restrictions).
  - *Dermal*: Ethoxyquin was evaluated for acute dermal toxicity in a GLP-compliant study conducted in a manner similar to OECD Guideline 402. Crl:CD®BR albino rats (5/sex/dose) were exposed to 2,000 mg/kg (1.98 mL/kg) ethoxyquin (purity not specified) on clipped, intact skin under semi-occlusive conditions for 24 hours, with a 14-day post-exposure observation period. There was no mortality and there were no treatment-related clinical observations, including effects on body weights, in this study. The test substance induced very slight to slight erythema on 8 animals and 10 sites had signs of desquamation. No edema or other treatment-related findings were observed. Dermal irritation was reversible by Day 12 or earlier, except in two females with observed desquamation. A dermal LD_{50} > 2,000 mg/kg was estimated (Klimisch score 2 – reliable with restrictions).

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

Ethoxyquin was assigned a score of Very High for systemic toxicity (single dose) based on a LOAEL of 100 mg/kg in an acute oral toxicity study in dogs, supported by Japan's GHS Category 1 classification based on the same critical study. GreenScreen® criteria classify chemicals as a Very High hazard for

systemic toxicity (single dose) when they are classified to GHS Category 1 and when oral LOAELs are no greater than 300 mg/kg (CPA 2018b). Confidence in the score is high as it is based on a reliable study and a NOAEL established by JMPR.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:*
        - GHS – Japan – H370 – Causes damage to organs [Specific target organs/systemic toxicity following single exposure – Category 1]
            - Category 1 classification based on a single dose oral toxicity study in dogs administered 50, 100, or 200 mg/kg test substance. There were no treatment-related effects on hematology, but higher serum bilirubin concentrations, lower urea nitrogen concentrations, and mild cholestasis in microscopic examinations were noted in both males and females in all treatment groups. Deposits of hepatocellular glycogen, in addition to cholestasis, were reduced in the dogs in the high dose group. Minimal cholestasis was observed in all treatment groups at the completion of the observation period. The Category 1 classification is based on treatment-related effects on the liver in dogs (NITE 2010).
- ECHA 2021a
    - *Oral*: Ethoxyquin (purity not specified) was evaluated for acute oral toxicity in a GLP-compliant study conducted in a manner similar to OECD Guideline 401. Albino rats (Crl:CD®BR) (5/sex/dose) were orally exposed by gavage to the test substance at 1,500, 1,950, and 2,535 mg/kg, followed by a 14-day observation period. Deaths of treated animals occurred withing 3 days of dosing, with the following mortalities: 2/10, 9/10, and 8/10 for the 1,500, 1,950, and 2,535 mg/kg dose groups, respectively. Clinical indicators of toxicity included ataxia in 24 animals, hypoactivity (21 animals), ocular discharge (16 animals), urogenital staining, hypothermia (cool to the touch, 13 animals), dried red material around the eyes, forelimbs, and nose, labored breathing (11 animals), prostrate positioning (10 rats), abnormal excretion (5 rats), and dried yellow material around the mouth. Surviving animals appeared normal, aside from the observation of dried yellow material around the mouth. There was no treatment-related effect on body weight. Histopathological examination revealed irritation in the gastrointestinal tract, and three animals exhibited hemorrhagic thymus glands. Dark red lungs and red fluid in the urinary bladder were observed in two rats. Enlarged cervical lymph nodes were noted in one animal and eye opacity in another animal. No other gross necropsy findings were observed in the animals that survived to planned euthanization (Klimisch score 2 – reliable with restrictions)
    - *Inhalation*: Ethoxyquin was evaluated for acute inhalation toxicity in a GLP-compliant study conducted in a manner similar to OECD Guideline 403. Crl:CD®BR rats (5/sex/dose) were exposed whole body to an aerosol of 1.97 mg/L ethoxyquin (purity not specified) (100 % of the particles < 10 microns and 13.4 % < 1.18 microns) for 4 hours, with a 14-day post administration observation period. One animal was sacrificed on Day 1 for humane reasons, while all other animals survived the dosing and observation periods. Clinical observations included salivation during dosing, wet and/or dried yellow material on external body surfaces, dried red material around the nose, and dried yellow material around the eyes. There were no other significant clinical observations or effects on body weight during the study; however, the study authors suggest that tremors in 6/10 animals following treatment might indicate neurotoxic potential of ethoxyquin (Klimisch score 2 – reliable with restrictions).

  - *Dermal*: Ethoxyquin was evaluated for acute dermal toxicity in a GLP-compliant study conducted in a manner similar to OECD Guideline 402. Crl:CD®BR albino rats (5/sex/dose) were exposed to 2,000 mg/kg (1.98 mL/kg) ethoxyquin (purity not specified) on clipped, intact skin under semi-occlusive conditions for 24 hours, with a 14-day post-exposure observation period. There was no mortality and there were no treatment-related clinical observations, including effects on body weights, in this study. The test substance induced very slight to slight erythema on 8 animals and 10 sites had signs of desquamation. No edema or other treatment-related dermal findings were observed. Dermal irritation was reversible by Day 12 or earlier, except in two females with observed desquamation (Klimisch score 2 – reliable with restrictions).
- JMPR 2005
  - *Oral:* In a GLP-compliant acute toxicity study, Beagle dogs (6/sex) received a single oral dose of ethoxyquin (purity 98.93%) in capsules at 0, 50, 100 or 200 mg/kg. Four animals/sex were sacrificed 24 hours afterwards, and the remaining 2/sex were observed for 14 days before sacrifice. Examinations included clinical observation, body weight, food consumption, hematology, serum chemistry, urinalysis, ophthalmology, gross pathology and histopathology. All dosed male groups and two highest dose female groups had increased mean serum alkaline phosphatase and alanine aminotransferase levels at the end of the 2-week recovery period. All dosed groups had higher total serum bilirubin concentrations and all dosed females had lower urea nitrogen at 24 hours post dosing. All dosed animals also had higher incidences of bilirubin in the urine. The effects on bilirubin were reversible, and decreased urea nitrogen was attributed to slight hepatic insufficiency by study authors. Histopathology findings at 24 hours post dosing included minimal to mild bile stasis in the liver in all dosed groups, which correlated with increased total bilirubin in serum. High dose animals had depleted hepatocellular glycogen, and one male had increased leukocytes in intrahepatic blood vessels and altered hepatocellular cytoplasm. Minimal bile stasis in the liver was also observed in recovery group in all dosed male groups and the two highest dose female groups. Study authors stated that the serum biochemistry changes indicative of liver effects measured at 50 mg/kg were minimal to mild in severity, and hence the toxicological significance was equivocal. JMPR did not consider these effects observed at 50 mg/kg to be toxicologically significant, and assigned a NOAEL of 50 mg/kg and LOAEL of 100 mg/kg for this study.

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

Ethoxyquin was assigned a score of High for systemic toxicity (repeated dose) based on a LOAEL of 2.5 mg/kg/day in a two-generation reproductive toxicity study in dogs and a LOAEL of 2 mg/kg/day in a subchronic oral toxicity study in dogs. Japan classified it to GHS Category 1 based on the same subchronic dog study. GreenScreen® criteria classify chemicals as a High hazard for systemic toxicity (repeated dose) when they are classified to GHS Category 1 (CPA 2018b). The confidence in the score is high as it is based on experimental data showing consistent markers of liver toxicity.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan – H372 – Causes damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure – Category 1]

- The Category 1 classification is based on consistent evidence of liver toxicity in oral toxicity studies in dogs and rats. In a 90-day oral toxicity in dogs, total bilirubin concentration and activity of alkaline phosphatase (ALP), glutamate pyruvate transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), and gamma-GT was increased, with hepatic changes, including increased pigment deposition, hepatocellular necrosis, cytoplasmic vacuolation, and bile-duct hyperplasia observed microscopically at 20 mg/kg/day and higher doses. An increase in ALP, mild pigment deposition, and hepatocellular necrosis was recorded at doses as low as 4 mg/kg/day. In a 28-day oral toxicity study in dogs, increased activity of serum enzymes indicative of liver damage and pigment deposition in the liver were observed at 25 mg/kg/day (with a dose conversion to 8.3 mg/kg/day for a 90-day study). In a 28-day oral toxicity study in rats, total bilirubin concentration was increased and gamma-GT at 250 mg/kg/day (converted to 77.7 mg/kg/day for a 90-day study) (NITE 2010).

- ECHA 2021a
  - *Oral:* In a previously described non-GLP, non-guideline (peer-reviewed) two-generation reproductive toxicity study that included developmental endpoints, male and female beagle dogs (F0: 5 males and 10 females/treatment group; F1: 8 males and 13 female pups/treatment group) were fed diets containing 0, 100, or 225 ppm ethoxyquin (purity not specified) (approximately 2.5 and 5.6 mg/kg/day, respectively) for a minimum of 82 days before pairing. For the F1 matings, 8 male and 13 female pups were fed diets containing 0, 100, or 225 ppm ethoxyquin from weaning until breeding at an age of 10-30 months (and the 2nd estrus cycle in females). Routine physical examinations were performed, and animals were regularly observed. Urine and blood samples were collected for hematology, clinical chemistry, and urinalysis from fasted adults before treatment and at the end of the F0 phase, at weeks 10, 23, 36, 49 and 62, and at the end of the F1 growth phase, and at the conclusion of the F1 mating phase. Ophthalmological examinations were carried out at the beginning and end of the F1 growth and matin phases. Mating, birth (whelping), and lactation indices were recorded. F1 adults and pups showing signs of toxicity were necropsied and tissues (not specified) from controls and F1 animals were evaluated histologically, as well as tissues (not specified) from F2 pups showing signs of toxicity. Livers and gall bladders of F1 adults at the low dose and adrenal glands and spleens from F1 adult females at the low dose were analyzed. Macroscopic and microscopic evaluations were carried out on F0 and F1 animals that died or were sacrificed due to clinical signs of toxicity. A statistically significant increase in serum alkaline phosphatase activity was observed in F0 males in the high dose group and in F0 females in the low and high dose groups. In the second parental generation (P1), at 225 ppm (approximately 5.6 mg/kg/day), there was an increase in the number of both male and female pups with a raw or red anus, dehydration, nasal discharge, excessive lachrymation, thinness, and pale gums. Nasal discharge and excessive lachrymation were also observed in the low dose group. Treatment related reductions in erythrocyte count, hematocrit, and hemoglobin were observed, and partial thromboplastin time were reduced in treated females. Serum activities of alkaline phosphatase, gamma-glutamyl transferase (GT), and alanine aminotransferase were increased in animals in the high dose group, indicating impaired liver function. In the F1 high dose mating group, statistically significant reductions in glucose, cholesterol, protein, albumin, and albumin:globulin ratio were observed, along with increases in total bilirubin concentration and in gamma-GT, alkaline phosphatase, and alanine aminotransferase activities. Increases in the absolute weights of

the spleen and testes and both organs relative to brain weight were observed in treated males. Lesions in the liver of one high dose male and in the cervical lymph nodes of two high dose females were noted. Pigment protoporphyrin IX was observed in the livers of 7/13 females at the low dose, 2/7 males at the high dose, and 10/11 females at the high dose. Fibrosis and hemorrhage frequencies were increased in the spleens of females in the high dose and there was also an increase in the incidence of pituitary cysts in animals in the high dose group. In the F1 generation, signs of clinical toxicity were observed in treated animals, including raw or red anus, dehydration, nasal discharge, and excessive lachrymation. A LOAEL of 2.5 mg/kg/day were assigned due to clinical signs of parental systemic toxicity, including excessive lachrymation and dehydration, and changes in clinical chemistry and pigment deposition in the liver of treated animals at the low dose in both the F0 and F1 generations (Klimisch score 2 – reliable with restrictions).

- *Oral:* Ethoxyquin was evaluated in a repeated dose subchronic oral toxicity study conducted according to EPA OPP 82-1 (GLP-compliant. Male and female Sprague-Dawley rats (10/sex/dose) were exposed to the test substance (purity not specified) by gavage in corn oil, once daily for 90 days at 0, 20, 40, 200, and 400 mg/kg/day. Animals were evaluated for cage side and clinical observations, body weight, food consumption, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, gross pathology, and histopathology. Yellow material on body surfaces, salivation, red material around the mouth, and brown material were observed on the urogenital area. Body weights were reduced in all of the dose groups for the duration of the study and body weight gain was slightly reduced in males in the 40 mg/kg/day dose group. Decreased red blood cell count, hemoglobin, and hematocrit means were reduced in the 200 and 400 mg/kg/day dose groups in both males and females, and an increased mean reticulocyte count was observed in the 200 mg/kg/day dose females and 400 mg/kg/day dose males and females. Clinical chemistry changes were observed in the 200 and 400 mg/kg/day dose animals, including increased serum protein levels and calcium means in females, and increased bilirubin, gamma-GT, cholesterol, and thyroid stimulating hormone (TSH) (in 200 mg/kg/day dose males, only). Mean thyroxine was reduced in the high dose males. Mean urea nitrogen was increased and mean glucose was decreased in the high dose groups and, although these changes slight, they were considered treatment related. Mean liver and kidney weights (absolute and relative to final body weight) were increased in the 200 and 400 mg/kg/day dose groups, reddened and/or enlarged thyroid glands in the 200 and 400 mg/kg/day dose groups, and “abnormal contents” in the urinary bladder were observed at the 400 mg/kg/day dose. Microscopically, there were treatment-related lesions in the kidneys of the 200 and 400 mg/kg/day dosed animals, which included nephropathy in the females and papillary necrosis with hyaline droplets in both males and females. A conservative NOAEL of 20 mg/kg/day for males was assigned based on decreased body weight gain and a NOAEL of 40 mg/kg/day for females. Treatment related effects were observed in clinical biochemistry, gross pathology, hematology, and non-neoplastic histopathology endpoints, as well as increases in liver and kidney weights and thyroid gland enlargement at the two higher doses (Klimisch score 2 – reliable with restrictions).
- *Oral:* In a repeated dose 28-day oral toxicity study conducted in a manner similar to OECD Guideline 407 (GLP-compliant), male and female Sprague-Dawley rats were administered daily doses of 0, 50, 250, 500, or 1,000 mg/kg/day ethoxyquin (purity not specified) in corn oil via gavage for 28 days. Animals were evaluated for cage side and clinical observations, body weight, food consumption, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, gross pathology, and histopathology. All of the top dose animals died between study days 2 and 3. Clinical signs of toxicity in the 1,000 mg/kg/day dose group

included salivation and yellow matting on the body. Treatment related gross lesions were observed in the gastrointestinal tract, kidneys, and lymph nodes and histopathological lesions were recorded in the 1,000 mg/kg/day group in the kidneys, liver, lungs, stomach, and lymph nodes. Cause of death in the 1,000 mg/kg/day dose group was described during histopathological examination as mucosal necrosis and ulceration of the forestomach, and kidney changes. Mean body weight gain was reduced in the 500 mg/kg/day dosed males up to week 1 and throughout the study in the 500 mg/kg/day dosed females. Red blood cell, hemoglobin, and hematocrit means were decreased in the 250 mg/kg/day dosed females and in the 500 mg/kg/day dosed males and females. There were also changes in serum chemistry parameters in the 250 and 500 mg/kg/day dosed animals. In the 500 mg/kg/day dosed groups, increased albumin, globulin, total protein, bilirubin, urea nitrogen, gamma-GT, cholesterol, calcium, phosphorus, and potassium means and decreased numbers for glucose and A/G ratio were observed. In the 250 mg/kg/day dosed groups, increased mean globulin, total protein, calcium, phosphorus, potassium, and cholesterol values and a decreased A/G ratio were recorded. Mean liver weights (absolute and relative to final body weight) in the 250 and 500 mg/kg/day dosed groups were increased relative to controls. Microscopic lesions in the kidneys, liver, lungs, stomach, and mediastinal lymph nodes in the 500 mg/kg/day dosed groups, with tubular dilatation in the kidneys, regeneration of the tubular epithelium, lymphocytic infiltration and hyperplasia of the urothelium observed. In two 500 mg/kg/day dosed females, hepatocellular swelling was recorded, and there were pulmonary lesions, including alveolar edema (one male) and hemorrhage (two males and three females) in the 500 mg/kg/day dosed groups. Stomach lesions, which included squamous cell hyperplasia, mononuclear leukocytic infiltration, and submucosal edema, were observed in one 500 mg/kg/day dosed female. Congestion of the mediastinal lymph node was observed in one 500 mg/kg/day dosed female. In the 250 mg/kg/day groups, treatment-related lesions in the kidney, including regeneration of the tubular epithelium in three males and renal tubular dilatation in two males were noted. A NOAEL of 50 mg/kg/day was assigned for this study (Klimisch score 2 – reliable with restrictions).

- EC 2008b
    - *Oral:* In a GLP-compliant subchronic toxicity study conducted according to FIFRA guideline 82-1, Beagle dogs (5/sex/dose) were exposed to ethoxyquin by capsules once daily at 0, 2, 4, 20 or 40 mg/kg/day for 90 consecutive days. High dose animals were exposed for only 7 weeks due to substantial systemic toxicity, and received empty capsules for the remainder of the study. Changes in clinical conditions, decreased body weight gain and food consumption, increased liver enzymes, discolored urine, dark livers, and microscopic liver lesions were found at 20 and 40 mg/kg/day. One high dose female was euthanized *in extremis*. High dose animals displayed substantial or complete recovery for all parameters measured at the end of the treatment free period. Effects observed at 4 mg/kg/day included increased alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase in two animals, mild to moderate endogenous liver pigmentation (low incidences), minimal hepatic cytoplasmic vacuolation, and minimal hepatocellular necrosis. EC report authors established a NOAEL of 2 mg/kg/day for this study based on the effects observed at the LOAEL of 4 mg/kg/day described above.
    - *Oral:* A 28-day dose range finding study was conducted for the 90-day study in dogs above. As the 90-day study is more robust and sufficient to assign the worst score with high confidence, ToxServices did not summarize this study.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
Ethoxyquin was assigned a score of Data Gap for neurotoxicity (single dose) based on insufficient data identified. Ataxia and hypoactivity that were reversible were observed at 1,500 – 2,535 mg/kg in an acute oral toxicity study at doses that caused deaths of some animals. Tremors were observed at an aerosol concentration of 1.97 mg/L which also caused sacrifice of one animal *in extremis.* Therefore, it is unclear if the observed effects are specific neurotoxicity or secondary to the acute general toxicity of the test compound. Additionally, the effects such as tremors are not consistent with transient narcotic effects (i.e., central nervous system depression), which warrant GHS Category 3 classification.

- 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 2021a
  - *Oral:* In the previously described GLP-compliant acute oral toxicity test conducted according to EPA OPP 81-1, male and female albino rats (5/sex/group) were administered gavage doses of undiluted test substance (purity not reported) at 1,500, 1,950 and 2,535 mg/kg. An observation period of 14 days followed the dosing. Deaths of treated animals occurred withing 3 days of dosing, with the following mortalities: 2/10, 9/10, and 8/10 for the 1,500, 1,950, and 2,535 mg/kg dose groups, respectively. Treatment caused clinical signs of neurotoxicity such as ataxia in 24 animals, and hypoactivity in 21 rats. These signs disappeared by day 7 or earlier (Klimisch Score 2, reliable with restrictions).
  - *Inhalation*: In the previously described GLP-compliant acute inhalation toxicity study conducted according to EPA OPP 81-3, male and female Crl:CD®BR rats (5/sex/dose) were exposed to the test substance (purity not reported) aerosol via whole body inhalation for 4 hours at a concentration of 1.97 mg/L. Treatment caused tremors in 6 of 10 animals following inhalation exposure. Study authors stated that this might suggest a neurotoxic potential and is considered to be in line with the frequent observation of ataxia in the acute oral toxicity study. However due to the study design (whole-body exposure), the toxic signs might be also (at least partly) due to oral intake of traces of the test substance from the fur and it cannot be certainly stated that they were exclusively caused by inhalation (Klimisch Score 2, reliable with restrictions).
- EC 2008c
  - Clinical signs observed in acute oral and inhalation studies may be associated with neurotoxicity of unknown origin at high doses.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): DG
Ethoxyquin was assigned a score of Data Gap for neurotoxicity (repeated 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.
- EC 2008c
  - Due to lack of specific measurements of neurotoxicity endpoints and the levels of ethoxyquin the brain in toxicokinetic studies, the neurotoxicity potential of ethoxyquin could not be determined. Nevertheless, delayed neuropathy is not a concern as ethoxyquin is not an organophosphorus compound that is known to induce delayed neurotoxicity.

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): M
Ethoxyquin was assigned a score of Moderate for skin sensitization based on being a weak sensitizer in an limited Buehler assay, being a GHS Category 1B sensitizer in the guinea pig maximization assay, and

positive reports in humans. GreenScreen® criteria classify chemicals as a Moderate hazard for skin sensitization when chemical warrants classification to GHS Category 1B (low to moderate frequency of occurrence) (CPA 2018b). The confidence in the score is high as it is based on consistent experimental data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:*
        - GHS – Japan – H317 – May cause an allergic skin reaction [Skin sensitizer – Category 1]
            - Category 1 classification based on contact dermatitis in mill workers exposed to animal feed flour and other positive results in patch tests in workers (further details not provided) (NITE 2010).
        - GHS – New Zealand – 6.5B (contact) – Contact sensitisers (Cat. 1)
            - Category 1 classification based on a weight of evidence from multiple sources (NZ EPA 2021).
- ECHA 2021a
    - Ethoxyquin (purity not specified) was tested in a GLP-compliant modified Buehler method dermal sensitization study in a manner similar to OECD Guideline 406. Male and female Dunkin-Hartley guinea pigs (6/sex/dose) were topically induced with the undiluted test substance, once per week for 3 weeks for a total of 3 induction exposures. The exposure periods each lasted 6 hours. Two weeks after the last induction exposure, animals were challenged with a topical application of test substance at a concentration of 50% and followed by another challenge at a concentration of 50%. Reactions to challenge and re-challenge were evaluated at 24 and 48 hours after exposure. Body weights and clinical observations were recorded before dosing and at the end of the study. A positive control group was induced and challenged with dinitrochlorobenzene (DNCB). No mortality, clinical findings, or body weight changes were observed in the study. Undiluted ethoxyquin induced 7 very slight reactions in the treated group following both the first and second induction doses. The 50% concentration of the test substance induced 10 very slight reactions at 24 hours in the treated group following the challenge dosing. After 48 hours, irritation included 9 sites with very slight reactions and 1 with a slight (grade 1) reaction. After the rechallenge with 50% test substance, there were 12 very slight reactions and by 48 hours, dermal irritation included 11 very slight reactions and 1 site with a slight (grade 1) reaction. Focal eschar was observed for one site at 24 and 48 hours. Four animals had desquamation at 48 hours after dosing. A "Sensitization Incidence Index" was calculated as 8% or 1/12 for the treated group following challenge dosing, and "Irritation Severity Indices" of 0.4 and 0.5 at 24 and 48 hours, respectively, were recorded. The Sensitization Incidence Index for the positive control was calculated to be 100% or 6/6 following challenge dosing with 0.1% DNCB, with Irritation Severity Indices of 1.8 and 1.5 at 24 and 48 hours, respectively. The authors of this study indicated that GHS criteria have not been met and ethoxyquin is not a skin sensitizer (Klimisch score 2 – reliable with restrictions).
        - EC report authors concluded that ethoxyquin may be a weak skin sensitizer based on the results of this study, but the severity of the responses does not warrant classification. However, the results were obscured by the difficulties in distinguishing irritation and sensitization reactions, and skin sensitization reactions may be underestimated due to very slight patchy erythema being described as equivocal (EC 2008c).

- EC 2008c
    - Dermal sensitization reactions were reported in workers handling fruit or animal diets containing ethoxyquin. Therefore, ethoxyquin is considered a human sensitizer.
    - In a GLP-compliant guinea pig maximization test conducted according to OECD Guideline 406, Hartley albino guinea pigs (20 total) were induced with six intradermal injections of an unspecified concentration of ethoxyquin (presumably unchanged 52% tradename product of ethoxyquin) and one week later induced epicutaneously with 52% ethoxyquin. Two weeks later, animals were epicutaneously challenged with 6% (highest non-irritating concentration) for 24 hours. Seven days later, animals were rechallenged epicutaneously with 3% or 1% ethoxyquin. 16/20 and 15/20 positive responses were recorded at 24 hours and 48 hours after the first challenge, respectively. 15/20 and 8/20 positive responses were recorded at 24 hours and 48 hours after the second challenge with 3% ethoxyquin, respectively. 2/20 positive reactions were recorded at both 24 hours and 48 hours after the second challenge with 1% ethoxyquin. The sham control animals had 3/10 and 1/10 positive responses 24 hours and 48 hours after the challenge, respectively. Study authors concluded that ethoxyquin was a dermal sensitizer.
        - Per GHS criteria (UN 2021), ≥30% responding at >1% intradermal induction dose corresponds to GHS Category 1B.

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): DG
Ethoxyquin was assigned a score of DG for respiratory sensitization based on lack of adequate data for this endpoint.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2021
    - Ethoxyquin does not contain any structural alerts for respiratory sensitization (Appendix I)
- No data were identified for the target compound for this endpoint. Therefore, ToxServices attempted to evaluate the respiratory sensitization potential of 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) according to ECHA's guideline (ECHA 2017), which states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). Ethoxyquin does not contain any structural alerts but is expected to be a skin sensitizer based on positive experimental and human data. According to the ECHA guidance, the positive skin sensitization results in animals and lack of structural alerts and evidence of respiratory sensitization indicate that there is insufficient positive data for the chemical to be classified as a respiratory sensitizer. However, the guidance requires negative skin sensitization data in order to conclude that the chemical is not a respiratory sensitizer. GreenScreen® criteria require negative data in order to assign a Low (i.e., a lack of alerts is not sufficient). Due to the positive predictions for skin sensitization and uncertainty regarding whether the mechanisms of sensitization could correspond to respiratory sensitization, a Data Gap was assigned.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): L
Ethoxyquin was assigned a score of Moderate for skin irritation/corrosivity based two negative skin irritation studies in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for skin

irritation/corrosivity when adequate data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on a reliable study on the neat test substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – New Zealand – 6.3B – Mildly irritating to the skin
      - This Pharos listing is incorrect as no classification was made for skin irritation in the New Zealand Environmental Protection Agency (NZ EPA)'s database (NZ EPA 2021).
- ECHA 2021a
  - Ethoxyquin (purity not specified) induced slight erythema on the clipped skin of New Zealand White rabbits in a GLP-compliant study conducted in a manner similar to OECD Guideline 404. Six animals were administered on clipped skin a dressing containing 0.5 mL test substance, under semiocclusive conditions, for 4 hours. Treated skin was examined 30-60 minutes, 24, 48, and 72 hours, and then daily through Day 7, after removal of the test substance for signs of erythema and edema. The test substance induced very slight erythema (a score of 0.33/1) on 5 animals which was reversible by Day 4 or earlier. The mean scores across 24, 48 and 72 hours for each of the 6 animals tested were 0, 0.33, 0.66, 0, 1 and 0, respectively. No edema or other dermal findings were recorded. Desquamation was noted on two sites (Klimisch score 2 – reliable with restrictions).
    - The effects observed in this study are not sufficient to classify ethoxyquin as a Category 3 skin irritant. The criteria for a GHS Category 3 skin irritant are mean value $\geqslant$ 1.5 - < 2.3 for erythema/eschar or for edema in at least 2 of 3 animals tested at 24, 48, and 72 hours after patch removal (UN 2021).
- EC 2008c
  - Ethoxyquin (52% purity) was not irritating in a GLP-compliant skin irritation study conducted in New Zealand white rabbits exposed to 0.5 mL on the intact skin for 4 hours. The mean 24, 48 and 72 hours erythema/edema scores were 0.67, 0.33 and 0.67 for each of the three animals tested. Therefore, the severity does not warrant GHS classification.

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

Ethoxyquin was assigned a score of Low for eye irritation/corrosivity based on slight skin irritation observed with the neat substance in a rabbit study that does not require GHS classification. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when adequate data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on a reliable study on the neat material.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – New Zealand – 6.4A – Irritating to the eye (Cat. 2A)
      - Based on a study reviewed by JMPR in albino rabbits, and based on information from a material safety data sheet.
    - GHS – Japan – H319 – Causes serious eye irritation [Serious eye damage/eye irritation – Category 2B]
      - Based on a study reviewed by JMPR that reported transient conjunctival redness and chemosis that were reversible within 4 days in rabbits.
- ECHA 2021a
  - A GLP-compliant ocular irritation test conducted according to EPA OPP 81-4 was

performed with New Zealand white rabbits (6 animals; sex not specified) administered 0.1 mL of undiluted ethoxyquin (purity not specified) for 96 hours without rinsing. Ocular reactions were recorded and scored 1, 24, 48, and 72 hours, followed by observations up to Day 4 in the absence of a reaction. The mean conjunctivae score was 0.444 and the effects were fully reversible within 96 hours. The mean chemosis score was 0.167 and the effects were fully reversible within 72 hours. There was no evidence of damage or a treatment-related effect on the iris or the cornea (Klimisch score 2 – reliable with restrictions).

- The effects observed in this study are not sufficient to classify ethoxyquin as a GHS Category 2 eye irritant. The criteria for a GHS Category 2 eye irritant are as follows: corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2, and/or chemosis ≥ 2 (UN 2021).

- EC 2008c
  - In a GLP-compliant ocular irritation study conducted according to OECD Guideline 405, New Zealand white rabbits received 0.1 mL 52% ethoxyquin in one eye without rinsing. The eyes were scored at 1, 24, 48 and 72 hours post-instillation. The scores for corneal opacity and iritis were 0 across all time points in all animals. The mean 24, 48 and 72 hours scores for conjunctival redness were 0.67, 1, and 1 for each of the 3 animals, and the mean scores for chemosis were 0. Study authors concluded that the test material was slightly irritating to the eye but did not warrant GHS classification.
    - The effects observed in this study are not sufficient to classify ethoxyquin as a GHS Category 2 eye irritant. The criteria for a GHS Category 2 eye irritant are as follows: corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2, and/or chemosis ≥ 2 (UN 2021).

Ecotoxicity (Ecotox)

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

Ethoxyquin was assigned a score of High for acute aquatic toxicity based on the most conservative EC_{50} of 2 mg/L in daphnia. GreenScreen® criteria classify chemicals as a High hazard for acute aquatic toxicity when acute aquatic toxicity values are >1 to 10 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan – H400 – Very toxic to aquatic life [Hazardous to the aquatic environment (acute) – Category 1]
      - Based on a 72-hour ErC_{50} of 0.70 mg/L for algae.
        - ToxServices was unable to locate this study.
    - GHS – New Zealand – 9.1B (fish) – Very ecotoxic in the aquatic environment
      - This Pharos listing is incorrect as no classification was made for acute aquatic toxicity in the New Zealand Environmental Protection Agency (NZ EPA)'s database (NZ EPA 2021).
    - GHS – New Zealand – 9.1C (crustacean) - Harmful in the aquatic environment
      - This Pharos listing is incorrect as no classification was made for acute aquatic toxicity in the New Zealand Environmental Protection Agency (NZ EPA)'s database (NZ EPA 2021).

- ECHA 2021a
  - 96-hour LC_{50} (*Oncorhynchus mykiss*, rainbow trout) = 18 mg/L (GLP-compliant, OECD

203) (Klimisch score 2 – reliable with restrictions)
    - 96-hour NOEC (clinical signs) (*O. mykiss*, rainbow trout) = 3.5 mg/L (GLP-compliant, OECD 203) (Klimisch score 2 – reliable with restriction)
    - 24-hour mortality EC_{50} (*Daphnia magna*) > 2.9 mg/L (GLP-compliant, OECD II A 8.3.1) (Klimisch score 2 – reliable with restrictions)
    - 48-hour mortality EC_{50} (*D. magna*) = 2 mg/L (GLP-compliant, OECD II A 8.3.1) (Klimisch score 2 – reliable with restriction)
    - 72-hour growth rate and biomass EC_{50} (*Pseudokirchneriella subcapitata*, green algae) (GLP-compliant, OECD 201) (Klimisch score 2 – reliable with restrictions):
        - 72-hour - EC_{50} – growth rate: > 16 mg/L
        - 72-hour - EC_{50} – biomass: 6.1 mg/L
        - 72-hour NOEC – biomass and growth rate: 2.3 mg/L

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *H*
Ethoxyquin was assigned a score of High for chronic aquatic toxicity based on a modeled chronic value of 0.91 mg/L in daphnia. GreenScreen® criteria classify chemicals as a High hazard for chronic aquatic toxicity when chronic aquatic toxicity values are between 0.1 and 1 mg/L (CPA 2018b). The confidence in the score is low as it is based on modeled 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.
    - *Other:*
        - GHS – Japan – H410 – Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) – Category 1]
- ECHA 2021a
    - 72-hour growth rate and biomass NOEC (*P. subcapitata*, green algae) (GLP-compliant, OECD 201) (Klimisch score 2 – reliable with restriction):
        - 72-hour NOEC – biomass and growth rate: 2.3 mg/L
- U.S. EPA 2017a
    - Ethoxyquin belongs to the neutral organics ECOSAR chemical class. The most conservative predicted chronic values (ChVs) are 1.15 mg/L in fish, 0.91 mg/L in daphnia, and 2.87 mg/L in green algae (Appendix J).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *H*
Ethoxyquin was assigned a score of High for persistence based on being predicted to be not readily biodegradable and partitioning to the soil with a half-life of 75 days. GreenScreen® criteria classify chemicals as a High hazard for persistence when they are not readily biodegradable and primarily partition to the soil with half-lives of > 60 to 180 days (CPA 2018b). The confidence in the score is low as it is based on modeled predictions.

- 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 2021a
    - Ethoxyquin readily hydrolyzed to form seven degradation products, including methylated, demethylated, and deethylated quinoline and dimeric ethoxyquin through intermolecular rearrangement and dimerization at 25°C and pH of 5, 7 and 9 in the dark, with dissipation

half-lives (DT50) of 3.7, 6.7 and 9.3 days at each pH, respectively. No additional details were provided for the identities of the degradants (Klimisch 2, reliable with restrictions).

- U.S. EPA 2017b
    - The BIOWIN modeling Ready Biodegradable Predictor indicates that 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) is not expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 80.6% will partition to soil with a half-life of 75 days, 18.1% will partition to water with a half-life of 37.5 days, and 1.28% will partition to sediment with a half-life of 337.5 days, and 0.0457 will partition to the air with a half-life of 1 hour (Appendix K).

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL
Ethoxyquin was assigned a score of Very Low for bioaccumulation based on a measured log K_{ow} value of 3.39 at pH 7 and an estimated BCF value of 129.3. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when log K_{ow} values are ≤ 4 and BAF and BCF values are ≤ 100 (CPA 2018b). The confidence in the score is high as it is based on a measured log K_{ow} value.

- 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 2021a
    - Ethoxyquin (99.2% purity) has a measured log K_{ow} of 3.39 at pH of 7, 3.18 at pH 5, and 3.18 at pH 9 (temperature not specified) as identified in a GLP-compliant shake-flask method conducted according to EU Method A.8 (Klimisch score 2 – reliable with restrictions).
- U.S. EPA 2017b
    - BCFBAF predicts a BCF of 58.23 L/kg wet-wt using the from regression-based method and a BCF of 129.3 for the upper trophic level using the Arnot-Gobas method, both based on a measured log K_{ow} of 3.18 at pH 5 (Appendix K).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): L
Ethoxyquin was assigned a score of Low for reactivity based on experimental data demonstrating that it is not explosive under shock, friction, and thermal parameters and that it lacks a structural alert for oxidizing properties. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when no GHS classification can be assigned for any of the GHS reactivity sub-endpoints (CPA 2018b). The confidence in the score was high as it is 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.
- ECHA 2021a
    - The explosive properties of ethoxyquin (99.2%) were evaluated in a GLP-compliant study conducted according to EU method A.14. Under shock, friction, and thermal parameters, the test substance was shown to have no explosive properties (Klimisch score 2 – reliable with restrictions).
    - 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin) has no chemical groups associated with oxidizing properties.
- Based on this data, ToxServices did not classify ethoxyquin as a reactive chemical based on GHS criteria (UN 2021).

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

Ethoxyquin was assigned a score of Low for flammability based on not being classified as a flammable liquid. GreenScreen® criteria classify chemicals as a Low hazard for flammability when they are not classified per GHS as flammable liquids or solids (CPA 2018b). The confidence in the score was high as it is based on a measured flash point for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - The flash point of ethoxyquin (98.6%) was evaluated in a GLP-compliant study conducted according to EU Method A.9. A flash point of 150 ± 2°C was determined.
    - According to GHS criteria, the flash point is above the GHS Guidance value for Category 4 flammable liquid (93°C); therefore, ethoxyquin is not classified per GHS.

Use of New Approach Methodologies (NAMs)[12] 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, respiratory sensitization, persistence, and bioaccumulation, and *in vitro* assays for endocrine activity and genotoxicity. NAMs are non-animal alternative that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

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

As shown in Table 5, Type I (input data) uncertainties in ethoxyquin's NAMs dataset include insufficient *in vivo* experimental or human data for carcinogenicity, endocrine activity, respiratory sensitization, chronic aquatic toxicity and persistence, and lack of validated test methods for respiratory sensitization. Ethoxyquin's Type II (extrapolation output) uncertainties include uncertain *in vivo* relevance of *in silico* predictions and *in vitro* high throughput receptor binding assays of endocrine activity, the limitation of *in vitro* genotoxicity assays in mimicking metabolic systems, the lack of applicability domains for ToxCast models for endocrine activity, the limitation of OECD Toolbox and Toxtree in identifying structural alerts without defining applicability domains, and OECD Toolbox not accounting for non-immunologic mechanisms of respiratory sensitization. Some of ethoxyquin's uncertainties can be alleviated by the use of *in vitro* and/or in combination with *in vivo* data, and ECHA's decision framework to evaluate respiratory sensitization.

Table 5: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses

Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: Limited experimental data are available. **Endocrine activity**: Limited *in vivo* experimental data are available. **Respiratory sensitization**: No experimental data are available, and there are no validated test methods. **Chronic aquatic toxicity:** Experimental data are only available for one trophic level. **Persistence:** No measured data are available.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity**: Toxtree only identifies structural alerts (SAs), and no applicability domain can be defined (Toxtree 2018). **Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation

[12] 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).

	system does not entirely mimic *in vivo* conditions[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 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, of 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] The *in vitro* SCE assay (as defined in OECD 479, a guideline deleted in 2014) detects reciprocal exchange of DNA without providing the underlying mechanism of action[17]. **Endocrine activity:** The *in vivo* relevance of *in silico* receptor binding activity prediction and *in vitro* high throughput receptor binding assays is unclear due to lack of sufficient data on toxicokinetics. ToxCast models have no defined applicability domain/do not report reliability of the predictions. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	Y	*In silico* modeling: VEGA/Toxtree/OncoLogic/OECD Toolbox/Danish QSAR
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay/*in vitro* UDS assay/ *in vitro* sister chromatid exchange assay

[13] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[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.
[17] https://www.oecd.org/env/ehs/testing/Draft_Intro_Genotoxicity%20TGs%20September%202014.pdf

Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays/ToxCast models/ 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	N	
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	Y	*In silico* modeling: ECOSAR
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 Ethoxyquin (CAS #91-53-2)

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	6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	91-53-2	*L*	L	*M*	*M*	M	M	vH	H	DG	DG	M	DG	L	L	H	*H*	*H*	vL	L	L

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	2

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

Table 6

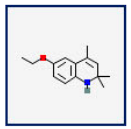
Chemical Name	Final GreenScreen® Benchmark Score
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	2

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

Table 5: Data Gap Assessment Table

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

APPENDIX C: Pharos Output for Ethoxyquin (CAS #91-53-2)



91-53-2

Ethoxyquin

ALSO CALLED (-)-Normetazocine, 1,2-Dihydro-2,2,4-trimethyl-6-ethoxyquinoline, 1,2-Dihydro-6-ethoxy-2,2,4-trimeth...

View all synonyms (47)

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Hazards | Properties | Functional Uses | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

		Group I Human					Group II and II* Human									Ecotox			Fate		Physical		Mult	Non-GSLT			
	GS Score	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
All Hazards	LT-P1	-	-	-	-	H-M	M	-	-	-	-	H	-	M	H	vH	-	M	-	-	-	-	vH	-	-	-	R

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Endocrine Activity	H-M	LT-P1	TEDX - Potential Endocrine Disruptors	Potential Endocrine Disruptor	

Acute Mammalian Toxicity	M	LT-UNK	EU - GHS (H-Statements)	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	+4
	H-M	LT-UNK	GHS - Australia	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	
	M	LT-UNK	GHS - Japan	H302 - Harmful if swallowed [Acute Toxicity (oral) - Category 4]	
	M	LT-UNK	GHS - New Zealand	6.1D (oral) - Acutely toxic	
	pC	NoGS	US EPA - OPP - Registered Pesticides	FIFRA Registered Pesticide	
Skin Sensitization	H	LT-UNK	GHS - Japan	H317 - May cause an allergic skin reaction [Skin sensitizer - Category 1]	+1
	H	LT-UNK	GHS - New Zealand	6.5B (contact) - Contact sensitisers (Cat. 1)	
Skin Irritation/Corrosivity	M	LT-UNK	GHS - New Zealand	6.3B - Mildly irritating to the skin	
Eye Irritation/Corrosivity	H	LT-UNK	GHS - New Zealand	6.4A - Irritating to the eye (Cat. 2A)	+1
	M	LT-UNK	GHS - Japan	H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2B]	
Acute Aquatic Toxicity	vH	LT-UNK	GHS - Japan	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	

Terrestrial Ecotoxicity	M	NoGS	GHS - New Zealand	9.3C - Harmful to terrestrial vertebrates	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-P1	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters	
Carcinogenicity,Mutagenicity/Genotoxicity Reproductive Toxicity, Developmental Toxicity, Acute Mammalian Toxicity, or System Toxicity/Organ Effects.	U	LT-UNK	Québec CSST - WHMIS 1988	Class D2B - Toxic material causing other toxic effects	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	vH	LT-UNK	GHS - Japan	H370 - Causes damage to organs [Specific target organs/systemic toxicity following single exposure - Category 1]	+1
	vH	LT-UNK	GHS - New Zealand	6.9A (oral) - Toxic to human target organs or systems (Cat. 1)	
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	GHS - New Zealand	9.1B (crustacean) - Very ecotoxic in the aquatic environment	+1
	U	LT-UNK	GHS - New Zealand	9.1C (fish) - Harmful in the aquatic environment	
T & P and/or B [(Chronic Aquatic Toxicity and sometimes Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	GHS - Japan	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
Systemic Toxicity/Organ Effects [Repeated Exposure] and/or Neurotoxicity [Repeated Exposure]	H	LT-UNK	GHS - Japan	H372 - Causes damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure - Category 1]	

Restricted Substance Lists (3)

- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- Food Contact Chemicals of Concern (FCCoCL): Food Contact Chemicals of Concern List (FCCoCL)
- Food Contact Chemicals of Concern (FCCoCL): Food Contact Chemicals of Concern List (FCCoCL) - TIER 3

APPENDIX D: Danish QSAR Carcinogenicity Results for Ethoxyquin (CAS #91-53-2)

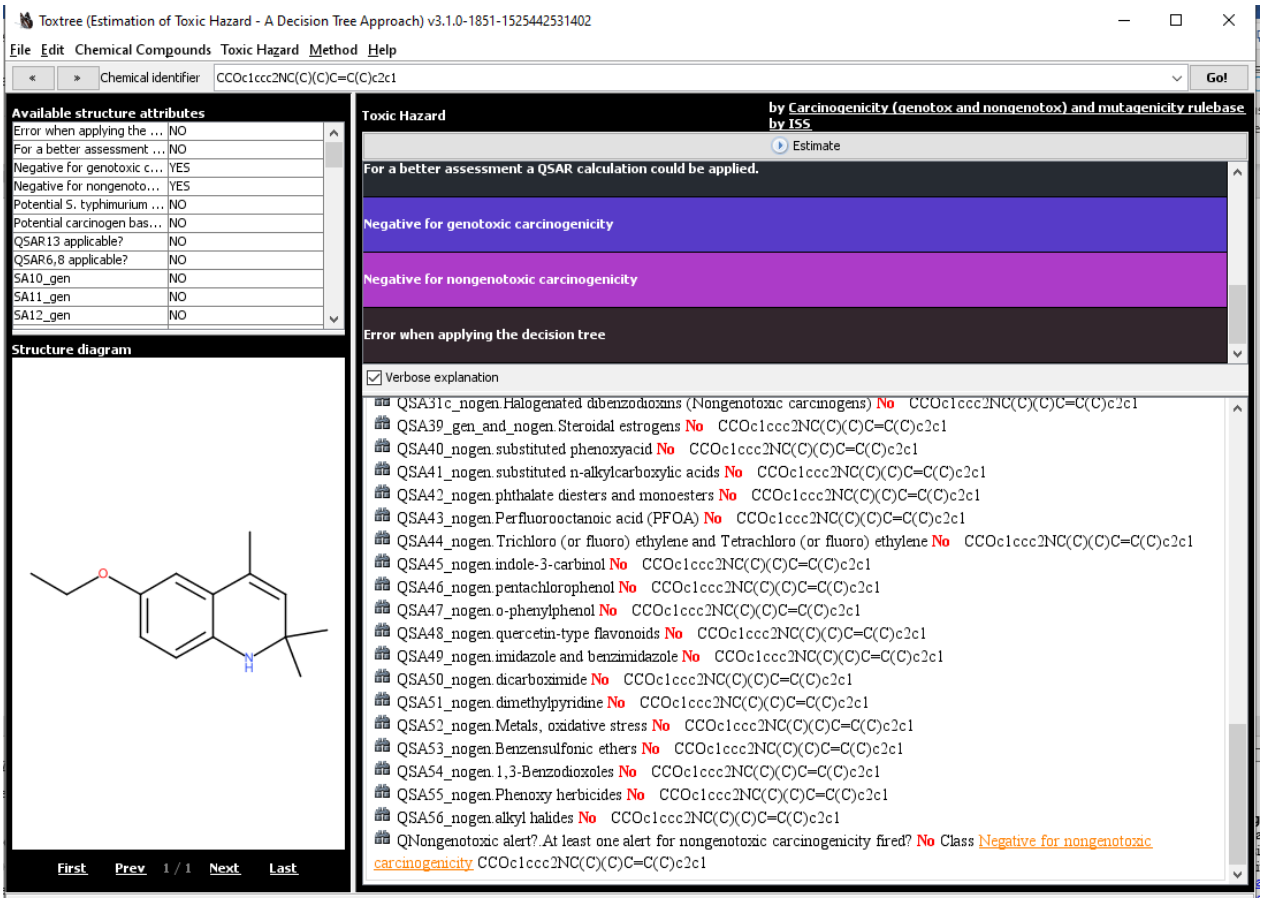
Carcinogenicity

	E Ultra	Leadscope
FDA RCA Cancer Male Rat	NEG_IN	NEG_OUT
FDA RCA Cancer Female Rat	NEG_IN	NEG_IN
FDA RCA Cancer Rat	NEG_IN	NEG_IN
FDA RCA Cancer Male Mouse	NEG_IN	NEG_IN
FDA RCA Cancer Female Mouse	NEG_IN	NEG_IN
FDA RCA Cancer Mouse	NEG_IN	NEG_IN
FDA RCA Cancer Rodent	NEG_IN	NEG_IN
Commercial models from CASE Ultra and Leadscope		
FDA RCA: Data from US Food and Drug Administration as part of Research Cooperation Agreement		

Carcinogenicity (genotox and nongenotox) alerts by ISS, alerts in:	
- parent only	No alert found
Oncologic Primary Classification, alerts in:	
- parent only	Not classified
OECD QSAR Toolbox v.4.2 profilers	
Profiler predictions are supporting information to be used together with the relevant QSAR predictions	

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Liver Specific Cancer in Rat or Mouse		INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
DTU-developed models					

APPENDIX E: Toxtree Carcinogenicity Results for Ethoxyquin (CAS #91-53-2)

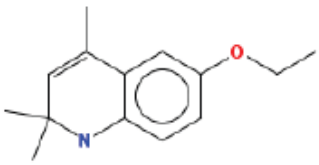


APPENDIX F: VEGA Carcinogenicity Results for Ethoxyquin (CAS #91-53-2)

VEGA Carcinogenicity model (CAESAR) 2.1.9 page 1

1. Prediction Summary

Prediction for compound Molecule 0

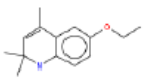


EXPERIMENTAL DATA

Experimental value is NON-Carcinogen. Model prediction is NON-Carcinogen (good reliability).

Compound: Molecule 0
Compound SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Experimental value: NON-Carcinogen
Predicted Carcinogen activity: NON-Carcinogen
P(Carcinogen): 0.213
P(NON-Carcinogen): 0.787
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

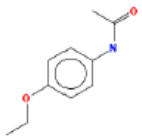
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 91-53-2
Dataset id: 301 (Training set)
SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Similarity: 1

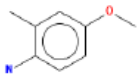
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #2

CAS: 62-44-2
Dataset id: 634 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

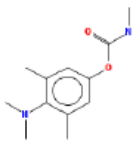
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #3

CAS: 102-50-1
Dataset id: 181 (Training set)
SMILES: O(c1ccc(N)c(c1)C)C
Similarity: 0.786

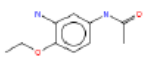
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #4

CAS: 315-18-4
Dataset id: 481 (Training set)
SMILES: O=C(Oc1cc(c(c(c1)C)N(C)C)C)NC
Similarity: 0.781

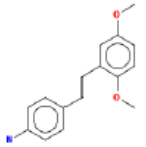
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 17026-81-2
Dataset id: 36 (Training set)
SMILES: O=C(Nc1ccc(OCC)c(N)c1)C
Similarity: 0.778

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



Compound #6

CAS: 5803-51-0
Dataset id: 255 (Training set)
SMILES: O(c2ccc(OC)c(C=Cc1ccc(N)cc1)c2)C
Similarity: 0.778

Experimental value: Carcinogen
Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 1
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 1
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.

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.

Model class assignment reliability
Pos/Non-Pos difference = 0.575
Explanation: model class assignment is well defined.

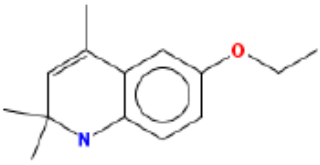
Neural map neurons concordance
Neurons concordance = 1
Explanation: predicted value agrees with experimental values of training set compounds laying in the same neuron.

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

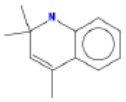


Prediction: Reliability:

Prediction is 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:
- 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

Compound: Molecule 0
Compound SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Experimental value: -
Predicted Carcinogen activity: NON-Carcinogen
Structural alerts: -
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

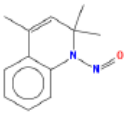
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 147-47-7
Dataset id: 707 (Training set)
SMILES: c1ccc2c(c1)NC(C=C2C)(C)C
Similarity: 0.876

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

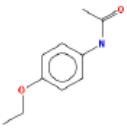


Compound #2

CAS: 29929-77-9
Dataset id: 562 (Training set)
SMILES: O=NN2c1ccccc1C(=CC2(C)C)C
Similarity: 0.861

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): SA21 Alkyl and aryl N-nitroso groups

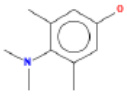


Compound #3

CAS: 62-44-2
Dataset id: 565 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

Experimental value: Carcinogen
Predicted value: Carcinogen

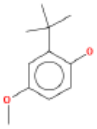
Alerts (not found in the target): SA28ter Aromatic N-acyl amine



Compound #4

CAS: 6120-10-1
Dataset id: 873 (Training set)
SMILES: Oc1cc(c(c(c1)C)N(C)C)C
Similarity: 0.799

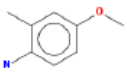
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 25013-16-5
Dataset id: 367 (Training set)
SMILES: Oc1ccc(OC)cc1C(C)(C)C
Similarity: 0.793

Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #6

CAS: 102-50-1
Dataset id: 172 (Training set)
SMILES: O(c1ccc(N)c(c1)C)C
Similarity: 0.786

Experimental value: Carcinogen
Predicted value: Carcinogen

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 Explanation: the predicted compound is outside the Applicability Domain of the model.
✔	**Similar molecules with known experimental value** Similarity index = 0.868 Explanation: strongly similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.495 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 = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

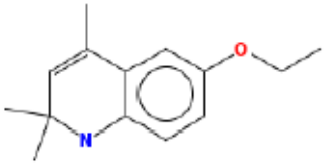
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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

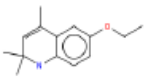


EXPERIMENTAL DATA

Experimental value is NON-Carcinogen. Model prediction is Carcinogen (low reliability).
The following relevant fragments have been found: Carcinogenity alert no. 28

Compound: Molecule 0
Compound SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Experimental value: NON-Carcinogen
Predicted Carcinogenic activity: Carcinogen
No. alerts for carcinogenicity: 1
Structural alerts: Carcinogenity alert no. 28
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

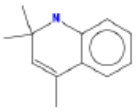


Compound #1

CAS: N.A.
Dataset id: 301 (Training set)
SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Similarity: 1

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 28

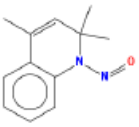


Compound #2

CAS: N.A.
Dataset id: 1507 (Training set)
SMILES: c1ccc2c(c1)NC(C=C2C)(C)C
Similarity: 0.876

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 28



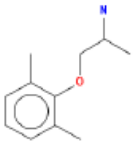
Compound #3

CAS: N.A.
Dataset id: 1468 (Training set)
SMILES: O=NN2c1ccccc1C(=CC2(C)C)C
Similarity: 0.861

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 28

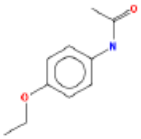
Alerts (not found in the target): Carcinogenity alert no. 8; Carcinogenity alert no. 48; Carcinogenity alert no. 49; Carcinogenity alert no. 50; Carcinogenity alert no. 55; Carcinogenity alert no. 63



Compound #4

CAS: N.A.
Dataset id: 1406 (Training set)
SMILES: O(c1c(cccc1C)C)CC(N)C
Similarity: 0.805

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

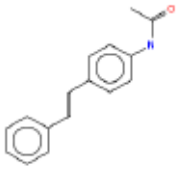


Compound #5

CAS: N.A.
Dataset id: 645 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

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



Compound #6

CAS: N.A.
Dataset id: 825 (Training set)
SMILES: O=C(Nc2ccc(C=Cc1ccccc1)cc2)C
Similarity: 0.794

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 25; Carcinogenity alert no. 31; Carcinogenity alert no. 34; Carcinogenity alert no. 89

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 = 1 Explanation: strongly similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0 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 = 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.

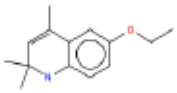
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on fragments/structural alerts:

Fragment found: Carcinogenity alert no. 28

Structural alert for carcinogenity defined by the SMARTS: Cc1ccccc1N

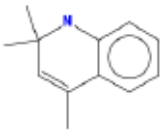
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: N.A.
Dataset id: 301 (Training set)
SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Similarity: 1

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

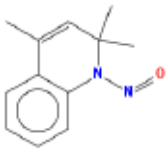
Alerts (found also in the target): Carcinogenity alert no. 28



CAS: N.A.
Dataset id: 1507 (Training set)
SMILES: c1ccc2c(c1)NC(C=C2C)(C)C
Similarity: 0.876

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 28



CAS: N.A.
Dataset id: 1468 (Training set)
SMILES: O=NN2c1ccccc1C(=CC2(C)C)C
Similarity: 0.861

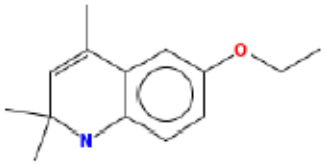
Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 28

Alerts (not found in the target): Carcinogenity alert no. 8; Carcinogenity alert no. 48; Carcinogenity alert no. 49; Carcinogenity alert no. 50; Carcinogenity alert no. 55; Carcinogenity alert no. 63

1. Prediction Summary

Prediction for compound Molecule 0

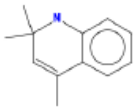


Prediction: Reliability:

Prediction is Carcinogen, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections.
The following relevant fragments have been found: Carcinogenity alert no. 20; Carcinogenity alert no. 42

Compound: Molecule 0
Compound SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Experimental value: -
Predicted Carcinogenic activity: Carcinogen
No. alerts for carcinogenicity: 2
Structural alerts: Carcinogenity alert no. 20; Carcinogenity alert no. 42
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

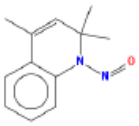


Compound #1

CAS: 147-47-7
Dataset id: 562 (Training set)
SMILES: c1ccc2c(c1)NC(C=C2C)(C)C
Similarity: 0.876

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42



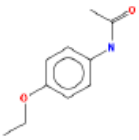
Compound #2

CAS: 29929-77-9
Dataset id: 472 (Training set)
SMILES: O=NN2c1ccccc1C(=CC2(C)C)C
Similarity: 0.861

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27; Carcinogenity alert no. 32

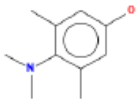


Compound #3

CAS: 62-44-2
Dataset id: 475 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 42

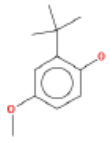


Compound #4

CAS: 6120-10-1
Dataset id: 774 (Training set)
SMILES: Oc1cc(c(c(c1)C)N(C)C)C
Similarity: 0.799

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 42

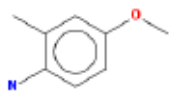


Compound #5

CAS: 25013-16-5
Dataset id: 298 (Training set)
SMILES: Oc1ccc(OC)cc1C(C)(C)C
Similarity: 0.793

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

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



Compound #6

CAS: 102-50-1
Dataset id: 139 (Training set)
SMILES: O(c1ccc(N)c(c1)C)C
Similarity: 0.786

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 42

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.917
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.842
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.

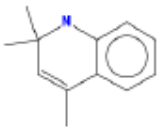
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

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

Fragment found: Carcinogenity alert no. 20

Structural alert for carcinogenity defined by the SMARTS: C=CCN

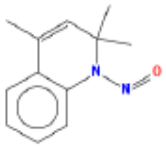
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: 147-47-7
Dataset id: 562 (Training set)
SMILES: c1ccc2c(c1)NC(C=C2C)(C)C
Similarity: 0.876

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42

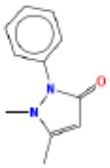


CAS: 29929-77-9
Dataset id: 472 (Training set)
SMILES: O=NN2c1ccccc1C(=CC2(C)C)C
Similarity: 0.861

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27; Carcinogenity alert no. 32



CAS: 60-80-0
Dataset id: 477 (Training set)
SMILES: O=C1C=C(N(N1c2ccccc2)C)C
Similarity: 0.747

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42

Alerts (not found in the target): Carcinogenity alert no. 27; Carcinogenity alert no. 28; Carcinogenity alert no. 32

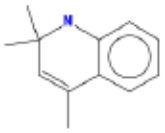
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

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

Fragment found: Carcinogenity alert no. 42

Structural alert for carcinogenity defined by the SMARTS: Nc1ccccc1

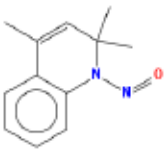
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: 147-47-7
Dataset id: 562 (Training set)
SMILES: c1ccc2c(c1)NC(C=C2C)(C)C
Similarity: 0.876

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42

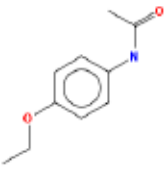


CAS: 29929-77-9
Dataset id: 472 (Training set)
SMILES: O=NN2c1ccccc1C(=CC2(C)C)C
Similarity: 0.861

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 42

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27; Carcinogenity alert no. 32



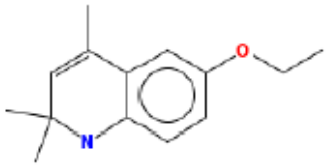
CAS: 62-44-2
Dataset id: 475 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 42

1. Prediction Summary

Prediction for compound Molecule 0

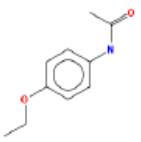


Prediction: Reliability:

Prediction is Carcinogen, but the result shows some critical aspects, which require to be checked:
- 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

Compound: Molecule 0
Compound SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Experimental value: -
Predicted Oral Carcinogenic class: Carcinogen
Reliability: the predicted compound could be out of the Applicability Domain of the model
Remarks:
none

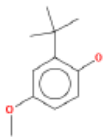
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 62-44-2
Dataset id: 254 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

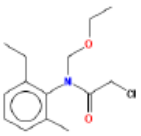
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #2

CAS: 25013-16-5
Dataset id: 51 (Test set)
SMILES: Oc1ccc(OC)cc1C(C)(C)C
Similarity: 0.793

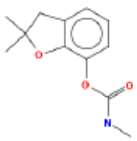
Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #3

CAS: 34256-82-1
Dataset id: 320 (Training set)
SMILES: O=C(N(c1c(cccc1CC)C)COCC)CCl
Similarity: 0.772

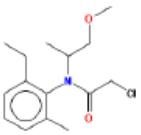
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #4

CAS: 1563-66-2
Dataset id: 371 (Training set)
SMILES: O=C(Oc2cccc1c2(OC(C)(C)C1))NC
Similarity: 0.771

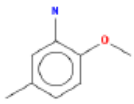
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 51218-45-2
Dataset id: 592 (Training set)
SMILES: O=C(N(c1c(cccc1CC)C)C(C)COC)CCl
Similarity: 0.768

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



Compound #6

CAS: 120-71-8
Dataset id: 81 (Training set)
SMILES: O(c1ccc(cc1(N))C)C
Similarity: 0.766

Experimental value: Carcinogen
Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	**Global AD Index** AD index = 0.752 Explanation: the predicted compound could be out of the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.797 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.504 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 = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

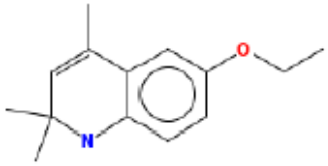
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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

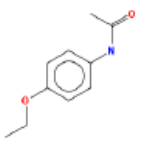


Prediction: Reliability:

Prediction is Carcinogen, but the result shows some critical aspects, which require to be checked:
- 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

Compound: Molecule 0
Compound SMILES: O(c1ccc2NC(C=C(c2(c1))C)(C)C)CC
Experimental value: -
Predicted Inhalation Carcinogenic class: Carcinogen
Reliability: the predicted compound could be out of the Applicability Domain of the model
Remarks:
none

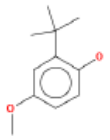
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 62-44-2
Dataset id: 217 (Training set)
SMILES: O=C(Nc1ccc(OCC)cc1)C
Similarity: 0.802

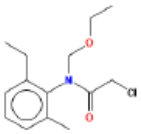
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #2

CAS: 25013-16-5
Dataset id: 44 (Training set)
SMILES: Oc1ccc(OC)cc1C(C)(C)C
Similarity: 0.793

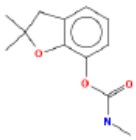
Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #3

CAS: 34256-82-1
Dataset id: 266 (Training set)
SMILES: O=C(N(c1c(cccc1CC)C)COCC)CCl
Similarity: 0.772

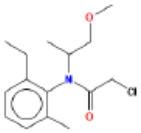
Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #4

CAS: 1563-66-2
Dataset id: 327 (Training set)
SMILES: O=C(Oc2cccc1c2(OC(C)(C)C1))NC
Similarity: 0.771

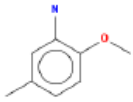
Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #5

CAS: 51218-45-2
Dataset id: 573 (Training set)
SMILES: O=C(N(c1c(cccc1CC)C)C(C)COC)CCl
Similarity: 0.768

Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #6

CAS: 120-71-8
Dataset id: 67 (Training set)
SMILES: O(c1ccc(cc1(N))C)C
Similarity: 0.766

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	**Global AD Index** AD index = 0.752 Explanation: the predicted compound could be out of the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.797 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
✖	**Accuracy of prediction for similar molecules** Accuracy index = 0.504 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 = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

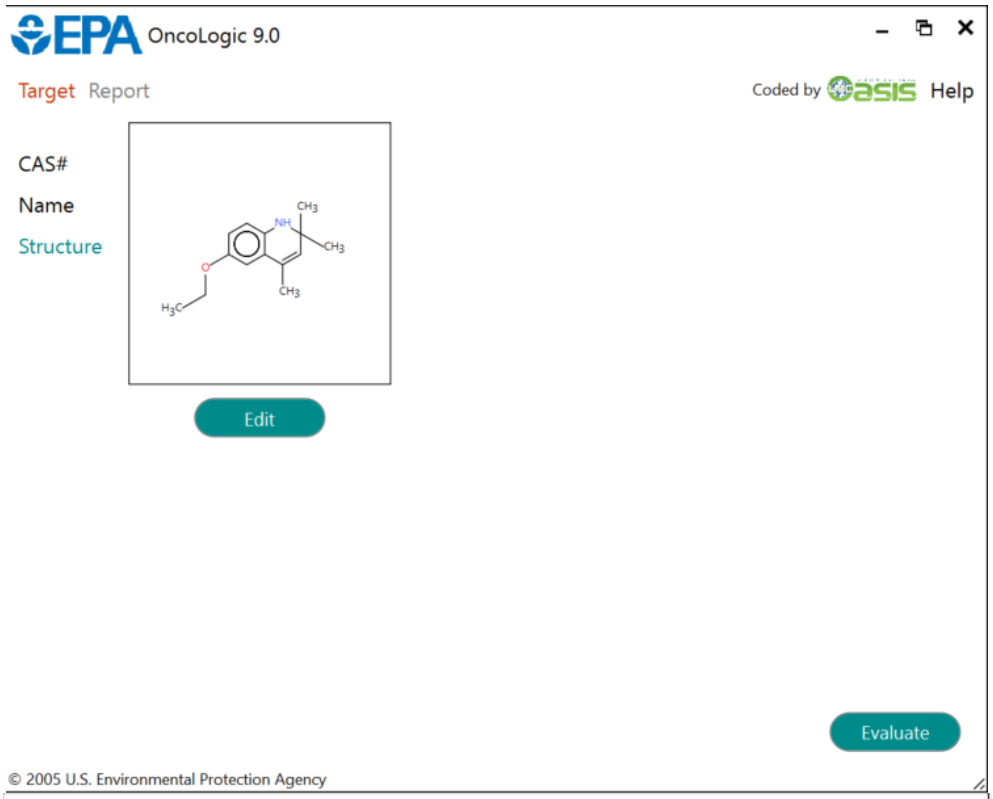
Symbols explanation:

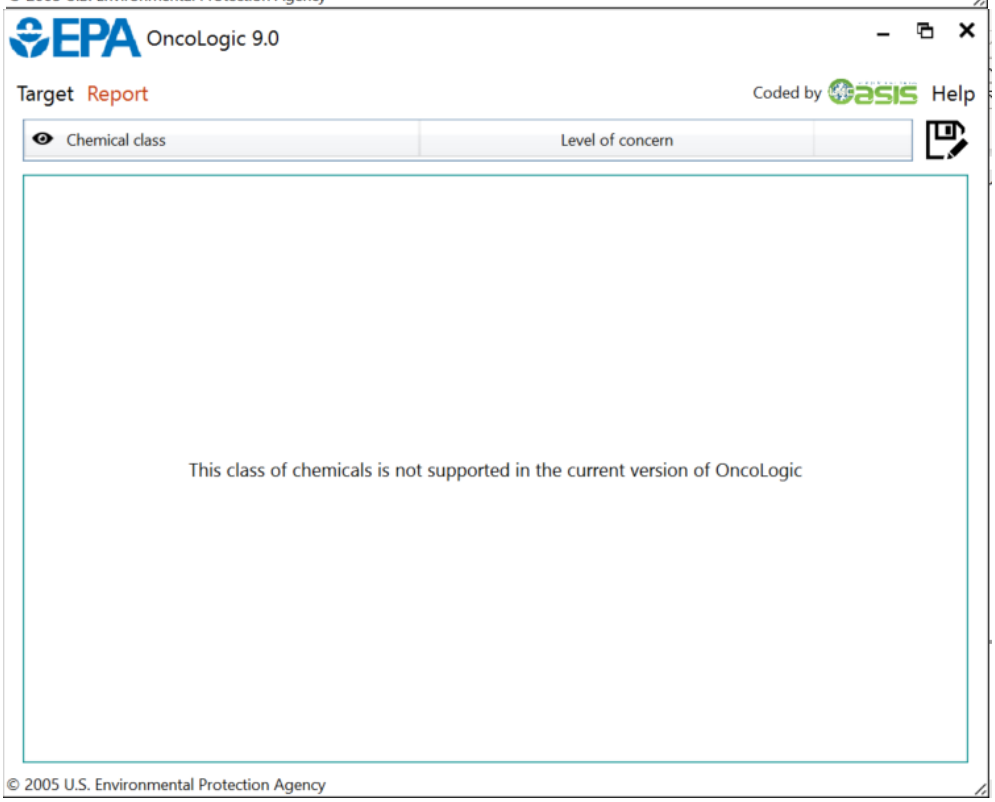
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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 G: Oncologic 9.0 Carcinogenicity Results for Ethoxyquin (CAS #91-53-2)





APPENDIX H: Danish QSAR Endocrine Activity Modeling for Ethoxyquin (CAS #91-53-2)

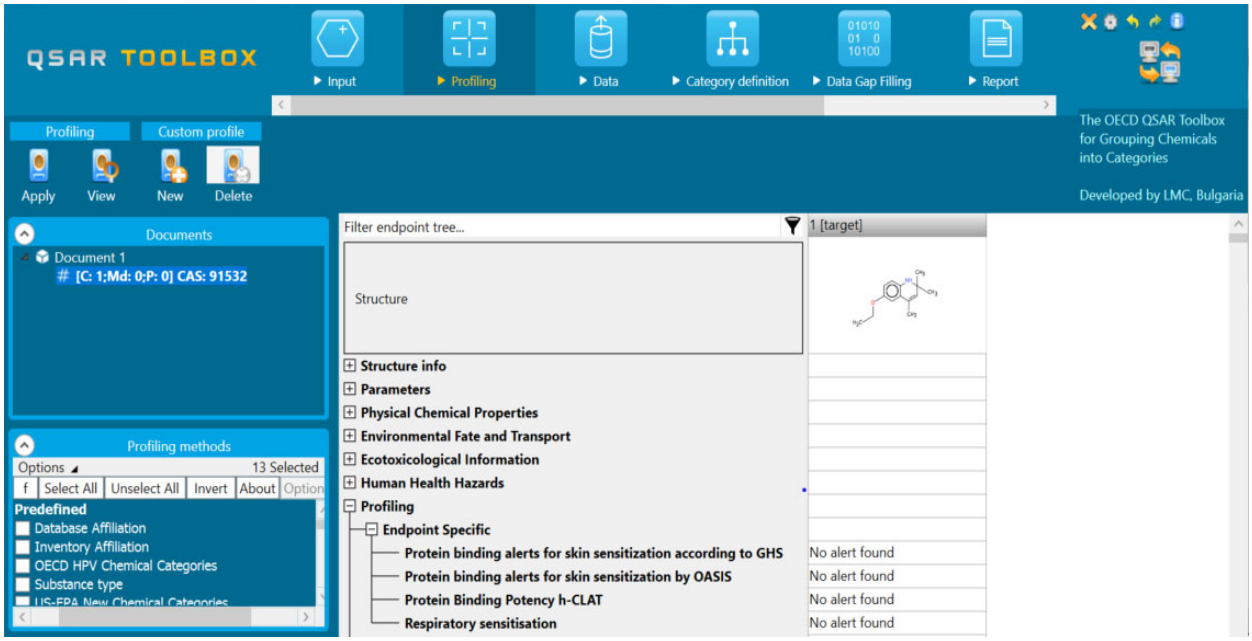
Endocrine and Molecular Endpoints

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor α Activation (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor Activation, CERAPP data (*in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human *in vitro*)		INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
Androgen Receptor Binding, CoMPARA data (*in vitro*)	NEG	N/A	N/A	INC_OUT	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)	NEG	N/A	N/A	INC_OUT	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)	POS	N/A	N/A	POS_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)	POS	N/A	N/A	POS_OUT	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L			34760	249.3903	1594.335
- µM			159955.8	1147.625	7336.687
- Positive for IC_{50} ≤ 10 µM					
- Positive for IC_{50} ≤ 100 µM					
- Domain		OUT	OUT	OUT	OUT

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L			7032.018	24.27167	7032.018
- µM			32359.38	111.6914	32359.38
- 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	NEG_IN	N/A
Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Pregnane X Receptor (PXR) Binding (Human *in vitro*)	N/A	INC_OUT	POS_OUT	INC_OUT	NEG_OUT
Pregnane X Receptor (PXR) Binding (Human *in vitro*) NEW		N/A	N/A	INC_OUT	N/A
Pregnane X Receptor (PXR) Activation (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (*in vitro*)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (*in vitro*)		N/A	N/A	INC_OUT	N/A
CYP3A4 Induction (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
DTU-developed models					

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; Moderate binder, OH grooup
- metabolites from Rat liver S9 metabolism simulator only	Strong binder, OH group; Moderate binder, OH grooup
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	

APPENDIX I: OECD QSAR Respiratory Sensitization Modeling Results for Ethoxyquin (CAS #91-53-2)



APPENDIX J: ECOSAR Modeling Results for Ethoxyquin (CAS #91-53-2)

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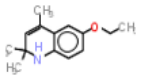
Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
91532	Quinoline, 6-ethoxy-1,2-dihydro-2,2,4-trimethyl-	O(c(ccc(NC(C=C1C)(C)C)c12)c2)CC

Structure



Details	
Mol Wt	217.31
Selected LogKow	3.39
Selected Water Solubility (mg/L)	60
Selected Melting Point (°C)	
Estimated LogKow	3.87
Estimated Water Solubility (mg/L)	45.29
Measured LogKow	
Measured Water Solubility (mg/L)	
Measured Melting Point (°C)	

Class Results:	

Neutral Organics

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	10.08	5	
Daphnid	48h	LC50	6.51	5	
Green Algae	96h	EC50	8.24	6.4	
Fish		ChV	1.15	8	
Daphnid		ChV	0.91	8	
Green Algae		ChV	2.87	8	

1 / 2

Class Results:	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish (SW)	96h	LC50	12.79	5	
Mysid	96h	LC50	3.71	5	
Fish (SW)		ChV	3.24	8	
Mysid (SW)		ChV	0.21	8	
Earthworm	14d	LC50	271.11	6	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported

APPENDIX K: EPI Suite™ Modeling Results for Ethoxyquin (CAS #91-53-2)

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

CAS Number: 91-53-2
SMILES : CCOc1ccc2NC(C)(C)C=C(C)c2c1
CHEM : 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline
MOL FOR: C14 H19 N1 O1
MOL WT : 217.31

---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): 3.18
Boiling Point (deg C) : ------
Melting Point (deg C) : -20.00
Vapor Pressure (mm Hg) : 0.000259
Water Solubility (mg/L): 60
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 3.87

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 316.84 (Adapted Stein & Brown method)
Melting Pt (deg C): 104.68 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 0.000795 (Modified Grain method)
VP (Pa, 25 deg C) : 0.106 (Modified Grain method)
BP (exp database): 123-125 @ 2 mm Hg deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 197.2
log Kow used: 3.18 (user entered)
melt pt used: -20.00 deg C

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 20.093 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.42E-007 atm-m3/mole (2.45E-002 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: 1.234E-006 atm-m3/mole (1.251E-001 Pa-m3/mole)
VP: 0.000259 mm Hg (source: User-Entered)
WS: 60 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 3.18 (user entered)
Log Kaw used: -5.005 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 8.185
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.3583
Biowin2 (Non-Linear Model) : 0.1886
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.3137 (weeks-months)
Biowin4 (Primary Survey Model) : 3.3495 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.2258
Biowin6 (MITI Non-Linear Model): 0.0428
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.2501
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): 0.0345 Pa (0.000259 mm Hg)
Log Koa (Koawin est): 8.185
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 8.69E-005
Octanol/air (Koa) model: 3.76E-005
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.00313
Mackay model : 0.0069
Octanol/air (Koa) model: 0.003

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 128.2352 E-12 cm3/molecule-sec
Half-Life = 0.083 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 1.001 Hrs
Ozone Reaction:
OVERALL Ozone Rate Constant = 13.650000 E-17 cm3/molecule-sec
Half-Life = 0.084 Days (at 7E11 mol/cm3)
Half-Life = 2.015 Hrs
Fraction sorbed to airborne particulates (phi):
0.00501 (Junge-Pankow, Mackay avg)
0.003 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 1269 L/kg (MCI method)

Log Koc: 3.104 (MCI method)
Koc : 497.3 L/kg (Kow method)
Log Koc: 2.697 (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 = 1.765 (BCF = 58.23 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.1986 days (HL = 1.58 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.112 (BCF = 129.3)
Log BAF Arnot-Gobas method (upper trophic) = 2.112 (BAF = 129.3)
log Kow used: 3.18 (user entered)

Volatilization from Water:
Henry LC: 1.23E-006 atm-m3/mole (calculated from VP/WS)
Half-Life from Model River: 700.8 hours (29.2 days)
Half-Life from Model Lake : 7768 hours (323.7 days)

Removal In Wastewater Treatment:
Total removal: 7.61 percent
Total biodegradation: 0.14 percent
Total sludge adsorption: 7.40 percent
Total to Air: 0.07 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**0.0457**	**1**	**1000**
Water	**18.1**	**900**	**1000**
Soil	**80.6**	**1.8e+003**	**1000**
Sediment	**1.28**	**8.1e+003**	**0**

Persistence Time: 1.05e+003 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0457	1	1000
Water	18.1	900	1000
water	(18.1)		
biota	(0.00137)		
suspended sediment	(0.0344)		
Soil	80.6	1.8e+003	1000
Sediment	1.28	8.1e+003	0

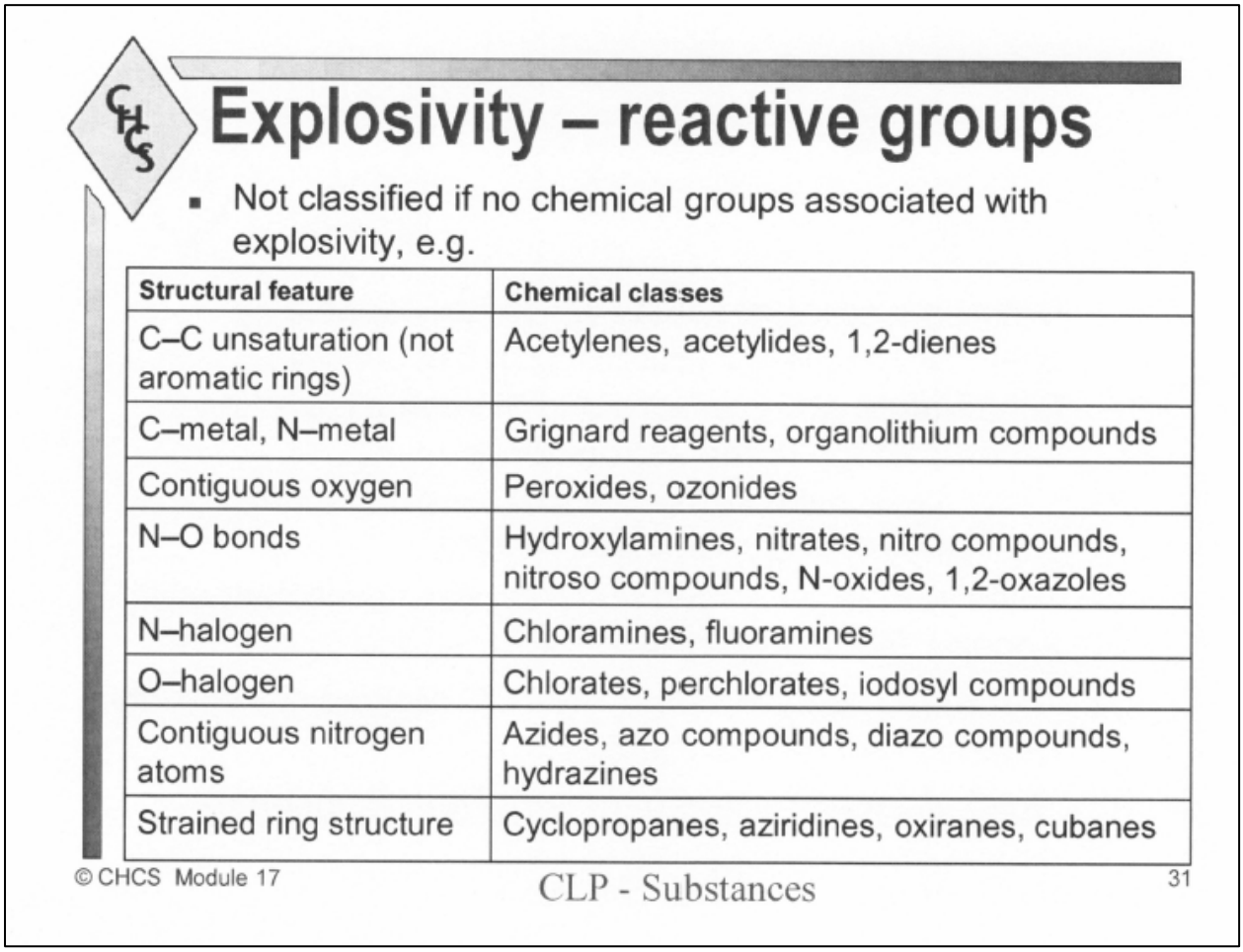
Persistence Time: 1.05e+003 hr

Level III Fugacity Model: (EQC Default)
Mass Amount Half-Life Emissions

	(percent)	(hr)	(kg/hr)
Air	0.0469	1	1000
Water	18.9	900	1000
water	(18.9)		
biota	(0.00143)		
suspended sediment	(0.0176)		
Soil	80.4	1.8e+003	1000
Sediment	0.648	8.1e+003	0
Persistence Time:	1.03e+003 hr		

APPENDIX L: 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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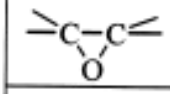
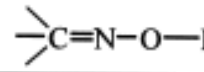
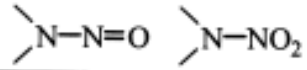
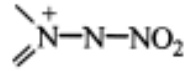
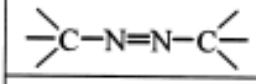
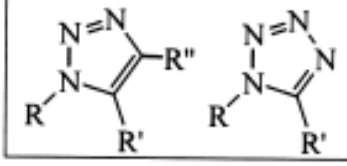
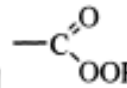
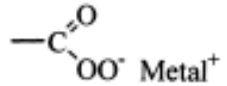
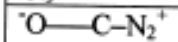
Explosivity – Full List

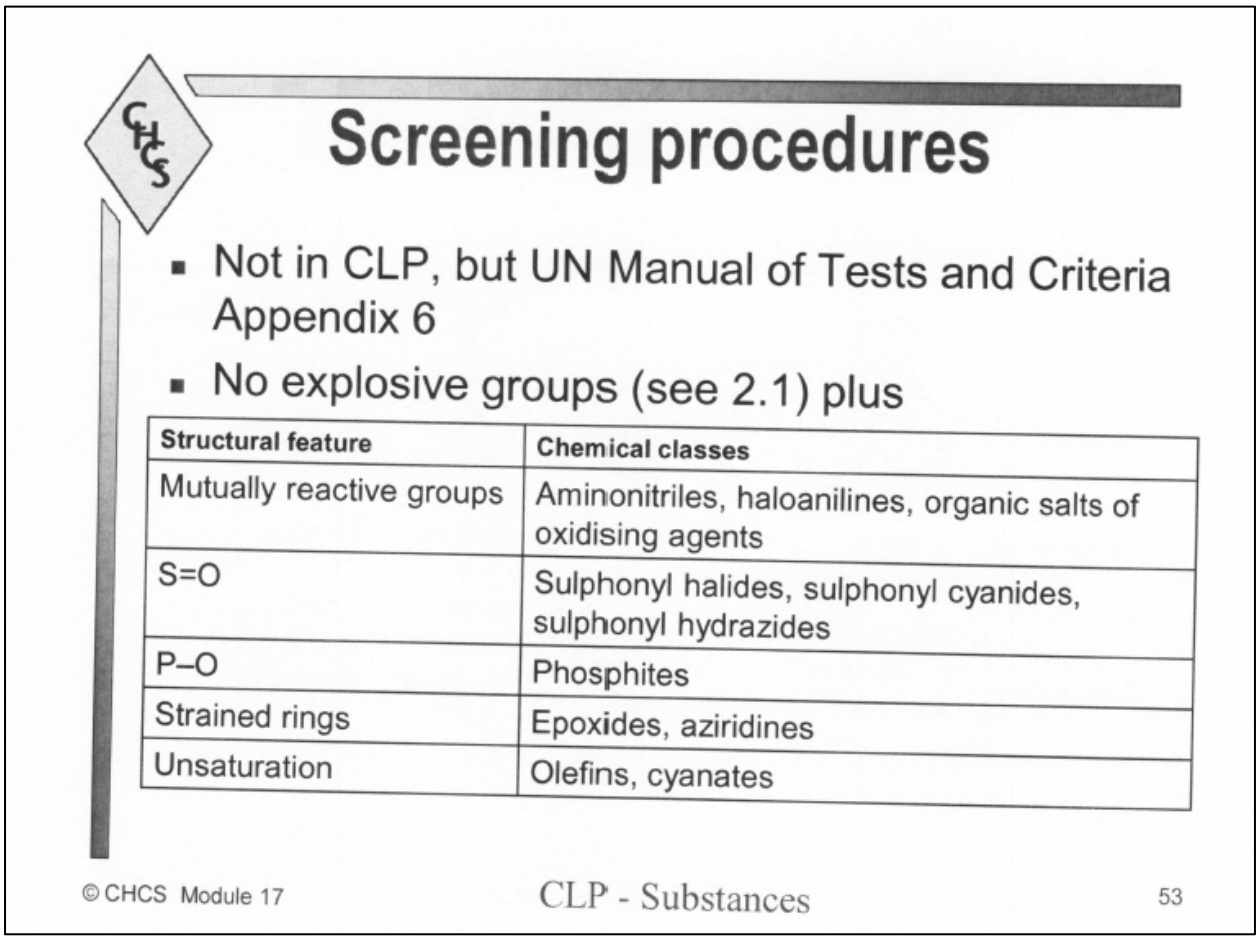
Table R.7.1-28 Chemical groups associated with explosive properties

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 M: Change in Benchmark Score

Table 6 provides a summary of changes to the GreenScreen® Benchmark™ for ethoxyquin. There has been one round of updates on GreenScreen® assessment, which does not affect its benchmark score.

Table 6: Change in GreenScreen® Benchmark™ for Ethoxyquin

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
October 15, 2021	BM-2	v. 1.4	New assessment
November 8, 2021	BM-2	v. 1.4	Minor updates based on Ecology's comments

Licensed GreenScreen® Profilers

Ethoxyquin GreenScreen® Evaluation Prepared by:

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