REACH

Phosphorodithioic acid, mixed O,O-bis(2-ethylhexyl and iso-Pr) esters, zinc salts

EC number: 272-723-1 | CAS number: 68909-93-3

Ecotoxicological information

Short-term toxicity to fish

001 Key | Experimental result

Administrative data

Endpoint:	short-term toxicity to fish
Type of information:	experimental study
Adequacy of study:	key study
Reliability:	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies:	other: Guideline study; restriction due to lack of analytical monitoring of test material

Data source

Reference

Reference Type:	study report
Title:	Unnamed
Year:	1986
Report date:	1986

Materials and methods

Test guideline

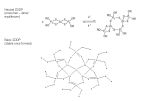
Qualifier:	according to guideline
Guideline:	OECD Guideline 203 (Fish, Acute Toxicity Test)
Deviations:	not specified

GLP compliance: yes

Test material

Test material information

Constituent 1



Reference substance name:	Phosphorodithioic acid, mixed O,O-bis(2-ethylhexyl and iso-Pr) esters, zinc salts
EC Number:	272-723-1
EC Name:	Phosphorodithioic acid, mixed O,O-bis(2-ethylhexyl and iso-Pr) esters, zinc salts
Cas Number:	68909-93-3
Molecular formula:	Too complex
IUPAC Name:	Phosphorodithioic acid, mixed O,O-bis(2-ethylhexyl and iso-Pr) esters, zinc salts

Specific details on test material used for the study:	Details on properties of test surrogate or analogue material (migrated information): PHYSICO-CHEMICAL PROPERTIES - Melting point: liquid at room temperature - Boiling point: decomposes before boiling - Vapour pressure: 0.0025 Pa at 25 deg. C - Henry's law constant (for volatile substances): low vapour pressure 0.0025Pa at 25 C - Water solubility (under test conditions): 1658 ppm at 22 deg. C - Solubility in organic solvents: no data - log Pow: 0.69 - pKa: no data - Base or acid catalysis of test material: no data - UV absorption: no data - Stability of test material at room temperature: stable - pH dependance on stability: hydrolytically stable at pH 4 and 7 for 5 days at 50 deg. C (OECD 111, Tier 1 preliminary study) OTHER PROPERTIES (if relevant for this endpoint) - Toxicity to microorganisms: EC50 > 10,000 mg/L (OECD 209) -Ready biodegradability: 1.5% in 28 d (OECD 301B)

Sampling and analysis

Analytical monitoring:	no
Details on sampling:	Concentrations: 0, 26, 64, 400 and 1000 mg/L Water Soluble Fractions

Test solutions

Vehicle:	no
Details on test solutions:	PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances) - Method: The test material solutions were prepared as Water Soluble Fractions (WSF). For each test concentration an appropriate aliquot by weight of the test material was added to dilution water in a glass aquarium. Each aquarium was fully covered and the solution was gently stirred overnight (24 hours) using a magnetic stirrer with a Teflon coated stir bar. Following an additional 24 hours of settling, the WSF of each concentration was separated from floating or settled material. Greater than 90% of the aged (approximately 24 hours old) test solution was replaced daily in each aquarium with freshly prepared test solution following the method described above. - Controls: untreated negative control; reference toxicant - Evidence of undissolved material (e.g. precipitate, surface film, etc): WSF were separated from floating or settled material and the solutions were observed to be colorless.

Test organisms

Test organisms (species):	Cyprinodon variegatus
Details on test organisms:	TEST ORGANISM - Common name: Sheepshead minnow - Strain: Cyprinodon variegatus - Source: A commercial supplier in Massachusetts - Age at study initiation (mean and range, SD): Test fish were 31 days old at the time of test initiation - Length at study initiation (length definition, mean, range and SD): mean =14.2 mm, range 11-18 mm, N=30

- Weight at study initiation (mean and range, SD): mean wet weight of 0.052 g; range 0.02 to 0.09 g; N=30

- Feeding during test: not fed during exposure

ACCLIMATION
- Acclimation period: Minimum of 7 days
- Acclimation conditions (same as test or not): Fish were held in glass aquaria or fiberglass holding tanks under a photoperiod of 16 hours light and 8 hours darkness. A closed loop recirculatng filtration system provided natural filtered (5 um) seawater with a salinity range of 32 to 36 ‰, a pH range of 7.5, and a dissolved oxygen concentration range of 96% of saturation. The temperature range in the holding trank was 23 to 24 degrees C.
- Type and amount of food: Fish were fed less than 48 hours old Artemia nauplii, ad libitum, daily until the day prior to testing
- Feeding frequency: ad libitum
- Health during acclimation (any mortality observed): There was no mortality during the seven days prior to testing

QUARANTINE (wild caught)
- Duration: not applicable
- Health/mortality: not applicable

Study design

Test type:	semi-static
Water media type:	saltwater
Limit test:	no
Total exposure duration:	96 h

Test conditions

Test temperature:	22 degrees C
pH:	No treatment related differences in pH. Mean pH values ranged from 7.6 to 7.8 in control and test solutions.
Dissolved oxygen:	No treatment related differences in DO. All solutions measured were greater than 60% of saturation and the range at the start of the test was from 6.9 to 7.8 mg/L
Salinity:	32%
Nominal and measured concentrations:	Nominal concentrations of 0, 26, 64, 160, 400, and 1000 mg/L WSF
Details on test conditions:	TEST SYSTEM - Test vessel: 18.9 L glass covered glass aquaria that contained 5 L of test solution - Type (delete if not applicable): covered - Material, size, headspace, fill volume: 5 L of test solution with a test solution depth of 6.0 cm with a surface area of 985 cm2. - Aeration: no auxiliary aeration - Type of flow-through (e.g. peristaltic or proportional diluter): - Renewal rate of test solution (frequency/flow rate): daily - No. of organisms per vessel: 10 - No. of vessels per concentration (replicates): 2 - No. of vessels per control (replicates): 2 - No. of vessels per vehicle control (replicates): - Biomass loading rate: Ten sheepshead minnows selected impartially from the holding tank were placed in each test aquaria within 10 minutes after the test solutions had been prepared. The resulting test organism loading concentration was 0.104 grams of biomass per liter of test solution. TEST MEDIUM / WATER PARAMETERS - Source/preparation of dilution water: The dilution was used was natural seawater collected from Cape Cod Canal, Bourne, Massachusetts. The seawater was filtered through a 5 um porosity polypropylene core filter and an activated carbon canister and stored for a period of 1 to 4 days prior to use. - Culture medium different from test medium: No difference, the water in the culture system was the same type of water used in the bioassay. Fish were acclimated to test conditions prior to the study - Intervals of water quality measurement: Daily for temperature, pH, DO, salinity OTHER TEST CONDITIONS - Adjustment of pH: none

- Photoperiod: 16 hours light and 8 hours dark
- Light intensity: 25 foot candles with a color rendering index greater than 91 by a combination of Sylvania Growlux and Cool White fluorescent bulbs.

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : All aquaria were examined after 0, 24, 48, 72, and 96 hours of exposure as follows: mortalities were recorded, dead fish were removed, and observations of the fish and physical characteristics of the test solutions were recorded.

TEST CONCENTRATIONS
- Justification for using less concentrations than requested by guideline: NA
- Range finding study
A preliminary study was conducted using 100% WSF at 10,000 mg/L nominal concentrations for a minimum of 48 hours and a maximum of 96 hours with daily renewal of the test and control solutions. This study was conducted with duplicate exposure of ten fish each and included a control exposure.
- Test concentrations: 100% WSF at 10,000 mg/L nominal concentrations
- Results used to determine the conditions for the definitive study: The 96 hour mortality of the fish tested at 100% Water Soluble Fraction was 100%. Based on this result, the definitive test was deemed necessary.

Reference substance (positive control):	yes
Remarks:	sodium lauryl sulfate

Results and discussion

Effect concentrations

Effect concentrations 1

Duration:	96 h
Dose descriptor:	LC50
Effect conc.:	46 mg/L
Nominal / measured:	nominal
Basis for effect:	mortality (fish)
Remarks on result:	other: 33 - 61 mg/L

Effect concentrations 2

Duration:	96 h
Dose descriptor:	other: NOEL
Effect conc.:	< 26 mg/L
Nominal / measured:	nominal
Basis for effect:	mortality (fish)

Details on results:	- Behavioural abnormalities: The NOEL was reported to be less than 26 mg/L, the lowest concentration tested, and this endpoint is based on observations of physical and behavioral abnormalities including lethargy, loss of equilibrium, and darkened pigmentation. - Any observations (e.g. precipitation) that might cause a difference between measured and nominal values: no insoluble materials were reported in the test solutions
Results with reference substance (positive control):	- Results with reference substance valid? Yes. A reference test with sodium lauryl sulfate was conducted with the test fish and the resulting 96 hour LC50 was 1.3 mg/L with a 95% confidence interval of 0.72 to 2.0 mg/L.
Reported statistics and error estimates:	The concentrations tested and the corresponding mortality data derived from the definitive toxicity test were used to estimate the medial LC50 values and 95% confidence intervals using standard statistical computations and software. The

statistical methods available were probit analysis, nonlinear interpolation, and moving average analysis.

Any other information on results incl. tables

Sublethal observations / clinical signs:

Table 1. Cumulative Mortality Data

WSF Test Concentration mg/L	Replicate	Cumulative Mortality (%) at Hour			
		24	48	72	96
1000	R1	100	100	100	100
	R2	100	100	100	100
400	R1	100	100	100	100
	R2	100	100	100	100
160	R1	50	80	100	100
	R2	50	90	90	90
64	R1	30	50	50	60
	R2	60	70	80	80
26	R1	0	0	0	0
	R2	0	40	40	40
0, Control	R1	0	0	0	0
	R2	0	0	0	0

Table 2. Statistical Analysis of Mortality Data

Exposure Period (hours)	LC50 (mg/L)	95% Confidence Limits (mg/L)
24	100	76 to 140
48	54	37 to 74
72	48	34 to 64
96	46	33 to 61

Justification for Read Across from Analogue EC 270-608-0

Common Manufacturing Process: The test substance (EC 272-723-1) and the analogue (EC 270-608-0) are produced under a common manufacturing process in which a phosphorodithioic acid ester intermediate, $(RO)_2PS_2H$, is produced by the reaction of phosphorus pentasulfide with a mixture of two alcohols of a similar class - branched alcohols containing C3 and C8 carbons (test substance) and C4 and C5 carbons (analogue). The intermediate is neutralized with zinc oxide to produce the final multicomponent substance.The reaction is performed in the presence of a highly refined base oil which accounts for 8 – 10 % of the final products.

Impurities: The level of impurities in the submission substance and the analogue is minimal (< 0.1 % wt and 0.09% wt, respectively). Impurities have been identified as residual, unreacted alcohols from the production of the phosphorodithioic acid ester intermediates (isopropyl and 2-ethylhexyl alcohols in the submission substance and isobutyl alcohol and pentyl alcohol isomers in the analogue).

Same Chemical Category: The submission substance (EC 272-723-1) and the analogue (EC 270-608-0), generically referred to as ZDDPs, have been shown to have sufficient structural similarities to be included in theZinc Dialkydithiophosphate Category (ZDDPs)in the United States Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program.

Structural Similarity: The primary feature accounting for the similarity of the test substance (EC 272-723-1) and the analogue (EC 270-608-0) is the common organometallic core structure consisting of a central zinc metal bonded to four alkyldithiophosphate esters (ligands) by coordinate covalent bonds -$Zn[(S_2P(OR)_2]_2$ Structural variations between the test substance and the analogue are related to the alkyl (R) groups of the alkyldithiophosphate ligands.

Both substances contain a distribution of several different zinc dialkyldithiophosphates (ZDDPs). The type and distribution of the zinc dialkyldithiophosphates is determined by the alcohol mixture and charge ratios of the alcohols used in the manufacturing process.

The submission substance (EC 272-723-1) is a mixture of ZDDPs containing all isopropyldithiophosphate ligands, all 2-ethylhexyldithiophosphate ligands, and those containing both isopropyldithiophosphate and 2-ethylhexylldithiophosphate ligands resulting in a multicomponent substance with a molecular weight range of 492 - 772 (for monomers).

The analogue (EC 270-608-0) is a mixture of ZDDPs containing all isobutyldithiophosphate ligands, all pentyldithiophosphate ligands, and components containing both isobutyldithiophosphate and isomeric pentyldithiophosphate ligands resulting in a multicomponent substance with a molecular weight range of 548 – 604 (for monomers).

Tanimoto Fingerprint (ToxMatch Version 1.06 software) gives a similarity index greater than 0.8 (values range from 0, no similarity to 1, identical). Peer reviewed literature indicates that values greater than 0.6 are significantly similar. DSSTox similarity was 80% between the submission substance and the analogue.

Similarity of Physicochemical Properties: In addition to the structural similarities, similar physicochemical properties further support the justification for read across from the analogue. Both the test substance and analogue have similar physical states, densities and the same order of magnitude of vapour pressures and partition coefficients (logPow). Both were shown to be hydrolytically stable at pH 4, 7 and 9 in an OECD 111 preliminary hydrolysis study.

In evaluating the evidence for read across, significant consideration was given to water solubility. Water solubility studies conducted on the test substance and the analogue show the lower molecular weight ZDDP monomers are preferentially dissolved in water with solubility decreasing with increasing alkyl chain lengths (molecular weights) of the alkyldithiophosphate ligands. In this respect, the water soluble composition of the test substance (EC 272-723-1) and the analogue (EC 270-608-0) are considered to be sufficiently close to reasonably expect a similar mode of action and toxicological effects in aquatic toxicity studies.

Data Matrix for Read Across from Analogue

Property	Analogue (Data Source)	Submission Substance (Target)
EC	270-608-0	272-723-1
CAS	68457-79-4	68909-93-3
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts	Phosphorodithioic acid, mixed O,O-bis(2-ethylhexyl and iso-Pr) esters, zinc salts
Physical	Viscous liquid	Viscous liquid
Boiling Point	Decomposes before boiling	Decomposes before boiling
Density @ 15.6 deg. C (ASTM D4052)	1.17 mg/L	1.15 mg/L

Vapour Pressure @ 25 C (EU method A.4)	0.0023 Pa	0.0019 Pa
Water Solubility @ 22 deg. C (OECD 105)	1658 mg/L	2111 mg/L
Identity of water soluble components (% = GC area %)	ZDDPs containing 76% isobutyl (C4) dithiophosphate ligands + 24% containing mixed isobutyl (C4) and pentyl (C5) dithiophosphate ligands	ZDDP containing isopropyl (C3) dithiophosphate ligands
Average molecular weight of water solubles (weighted average based on GC peak area %)	562	492
Partition Coefficient, logPow (OECD 107)	0.69	0.84
Hydrolysis as a function of pH (OECD 111, Tier 1 preliminary study at pH 4,7 and 9)	Hydrolytically stable at pH 4, 7 and 9	Hydrolytically stable at pH 4, 7 and 9

Applicant's summary and conclusion

Validity criteria fulfilled:	yes
Conclusions:	The acute toxicity of the test material to the saltwater fish, Cyprinodon variegatus, was investigated and resulted in a 96 hour LC50 of 46 mg/L and a NOEL of less than 26 mg/L.
Executive summary:	Introduction. A study was performed to assess the acute toxicity of the test material to sheepshead minnow under static renewal test conditions using OECD 203 Guidelines. Methods. Following preliminary range finding studies fish were exposed in groups of ten to water soluble fractions of the test material at nominal test concentrations of 0, 26, 64, 160, 400, and 1000 mg/L. The test was conducted under static renewal conditions for 96 hours at a temperature of 22 degrees C. The number of fish mortalities and any sublethal effects were recorded after 24, 48, 72, and 96 hours. Results. The acute toxicity of the test material to the saltwater fish,Cyprinodon variegatuswas investigated and resulted in a 96 hour LC50 of 46 mg/L. A reference toxicity test was conducted with sodium lauryl sulfate resulting in a 96 hour LC50 of 1.3 mg/L.