CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Chemical name:

Acetone oxime

EC Number: **204-820-1**

CAS Number: **127-06-0**

Index Number: **-**

Contact details for dossier submitter:

Environment Agency Austria, Spittelauer Lände 5, A-1090 Vienna

on behalf of the Austrian Competent Authority (Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology. Radetzkystraße 2, 1030 Vienna, Austria)

Version number: 02 **Date: April 2020**

CONTENTS

1 IDENTITY OF THE SUBSTANCE 1

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 1
1.2 COMPOSITION OF THE SUBSTANCE 2

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 3

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 3

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 5

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 5

5 IDENTIFIED USES 5

6 DATA SOURCES 6

7 PHYSICOCHEMICAL PROPERTIES 6

8 EVALUATION OF PHYSICAL HAZARDS 7

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 7

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 9

10 EVALUATION OF HEALTH HAZARDS 11

10.1 ACUTE TOXICITY - ORAL ROUTE 11
10.2 ACUTE TOXICITY - DERMAL ROUTE 11
10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity 12
10.2.2 Comparison with the CLP criteria 13
10.2.3 Conclusion on classification and labelling for acute dermal toxicity 13
10.3 ACUTE TOXICITY - INHALATION ROUTE 13
10.4 SKIN CORROSION/IRRITATION 13
10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation 14
10.4.2 Comparison with the CLP criteria 14
10.4.3 Conclusion on classification and labelling for skin corrosion/irritation 14
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 14
10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation 15
10.5.2 Comparison with the CLP criteria 16
10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation 17
10.6 RESPIRATORY SENSITISATION 17
10.7 SKIN SENSITISATION 17
10.7.1 Short summary and overall relevance of the provided information on skin sensitisation 18
10.7.2 Comparison with the CLP criteria 19
10.7.3 Conclusion on classification and labelling for skin sensitisation 20
10.8 GERM CELL MUTAGENICITY 20
10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity 26
10.8.2 Comparison with the CLP criteria 28
10.8.3 Conclusion on classification and labelling for germ cell mutagenicity 30
10.9 CARCINOGENICITY 30
10.9.1 Short summary and overall relevance of the provided information on carcinogenicity 33
10.9.2 Comparison with the CLP criteria 34
10.9.3 Conclusion on classification and labelling for carcinogenicity 36
10.9.4 Specific concentration limit (SCL) 36
10.10 REPRODUCTIVE TOXICITY 36

10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE....37
10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure....39
10.11.2 Comparison with the CLP criteria....40
10.11.3 Conclusion on classification and labelling for STOT SE....41
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE....41
10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure....42
10.12.2 Comparison with the CLP criteria....45
10.12.3 Conclusion on classification and labelling for STOT RE....46
10.13 ASPIRATION HAZARD....46
11 EVALUATION OF ENVIRONMENTAL HAZARDS....46
12 EVALUATION OF ADDITIONAL HAZARDS....46
13 ADDITIONAL LABELLING....46
14 REFERENCES....47
15 ANNEX I: READ-ACROSS JUSTIFICATION....50
15.1 RELIABILITY AND ADEQUACY OF THE SOURCE STUDIES USED FOR READ-ACROSS....51
15.2 IDENTITY AND CHARACTERISATION OF THE SOURCE SUBSTANCES....51
15.3 LINK OF STRUCTURAL SIMILARITIES AND DIFFERENCES WITH THE PROPOSED PREDICTION (ANALOGUE APPROACH):....53
15.4 BIAS THAT MAY INFLUENCES THE PREDICTION....54
15.5 HYPOTHESIS FOR THE ANALOGUE APPROACH....55

ABBREVIATIONS

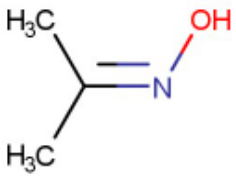
ALP	Alkaline Phosphatase
ATE	Acute Toxicity Estimate
CHO	Chinese Hamster Ovary
CSR	Chemical Safety Report
bw	Body weight
CAS	Chemical Abstract Service
Drg	Danger
d	Day
DEN	Diethylnitrosamine
DMEL	Derived Minimal Effect Level
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleic acid
DNEL	Derived no effect level
ECHA	European Chemical Agency
ESR	Electron Spin Resonance
FCA	Freund's Complete Adjuvant
GPMT	Guinea pig maximisation test
HCA	Hexyl cinnamic aldehyde (CAS No 101-86-0)
HLN	Hyperplastic liver nodules
HGST	Human glutathione S-transferase
i.p.	intraperitoneal
Kow	Partition coefficient octanol/water
LLNA	Local Lymphnode Assay
LOAEL	Lowest Observed Adverse Effect Level
MEST	Mouse Ear Swelling Test
MEKO	Methyl Ethyl Ketoxime = Butanone oxime
MI	Mitotic Index
m/f	male/female
NOAEL	No Observed Adverse Effect Level
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCE	Normochromatic erythrocytes
NOAEL	No Observed Adverse Effect Level
NO	Nitric oxide

NO^{2-}	Nitrogen dioxide
2-NP	2-Nitropropane
NTP	National Toxicology Program (https://ntp.niehs.nih.gov/)
n.r.	not reported
OSV	Ovine seminal vesicle
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocytes
P2-N	Propane 2-nitronate
RDT	repeated dose toxicity
RNA	ribonucleic acid
RTG	relative total growth
RS	relative survival
RBC	Red blood cells
SCE	Sister chromatid exchange
SD rat	Sprague Dawley rat
SMART	Somatic mutation and recombination test
TD50	Tumorogenic Dose (TD), which would induce tumors in half the test animals at the end of a standard lifespan for the species
TK	Toxicokinetic
UVCB	Chemical substances of unknown or variable composition

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	N-(propan-2-ylidene)hydroxylamine
Other names (usual name, trade name, abbreviation)	2-propanone, oxime propan-2-one oxime 2-(hydroxylimino)propane
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	204-820-1
EC name (if available and appropriate)	acetone oxime
CAS number (if available)	127-06-0
Other identity code (if available)	-
Molecular formula	C3H7NO
Structural formula	 **(source:** European Chemicals Agency, http://echa.europa.eu/**)**
SMILES notation (if available)	CC(=NO)C
Molecular weight or molecular weight range	73.09
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.2 Composition of the substance

Acetone oxime is a mono-constituent substance.

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP) of registrants
Acetone oxime EC 204-820-1 CAS 127-06-0	confidential	-	Flam. Solid 1, H228 Acute Tox. 4, H312 Eye Dam. 1, H318 Skin Sens. 1B, H317 Carc. 2, H351

Impurities not relevant for classification.

Information on the test substances (if available) are given in the study descriptions.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	606-RST-VW-Y	acetone oxime	204-820-1	127-06-0	Carc. 1B Acute Tox. 4 STOT SE 3 STOT RE 2 Eye Dam. 1 Skin Sens. 1B	H350 H312 H336 H373 (blood system) H318 H317	GHS08 GHS07 GHS05 Dgr	H350 H312 H336 H373 (blood system) H318 H317		dermal: ATE = 1100 mg/kg bw	

Table 4: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	Acute Tox. 4, H312	Yes
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Eye Dam. 1, H318	Yes
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	Skin Sens. 1B, H317	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	Carc. 1B, H350	Yes
Reproductive toxicity	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure	STOT SE 3, H336	Yes
Specific target organ toxicity-repeated exposure	STOT RE 2, H373	Yes
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	*hazard class not assessed in this dossier*	No
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has no harmonized classification so far.

The substance has 114 C&L notifications with self-classifications (summary) as Flam. Sol. 1, H228 or Flam Sol. 2, H228; Acute Tox. 4, H302; Acute Tox. 4, H312; Eye Dam. 1, H318; Skin Sens. 1B, H317 or Skin Sens 1, H317; Carc. 2, H351; STOT RE 2, H373 (red blood cells) [ECHA dissemination site accessed December 2020].

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Further detail on need of action at Community level:

The proposed classification and labelling of acetone oxime for carcinogenicity and narcotic effects is based on data on the substance and studies from read-across from butanone oxime. For mutagenicity also information from WASOX-MMAC2 (CAS 797751-43-0) and WASOX-VMAC2 (CAS 797751-33-0) is used. A read-across justification is provided in Annex I to the CLH report.

Harmonized classification for other endpoints based on data with acetone oxime is also proposed due to differences in self-classification and disagreement by DS with current self-classification of registrants.

5 IDENTIFIED USES

Table 5: The following uses are indicated at ECHA dissemination site [accessed December, 2020]:

Categories	Use(s)	Technical function
Manufacture	Manufacture of acetone oxime	-
Formulation	Formulation in preparations, (re-) packaging and distribution	-
Uses at industrial sites	Coatings/printing inks (PC 9a, 18) Uses in laboratories (PC 21) Intermediate Use as an intermediate for manufacture of silicon sealants (PC 1, 19)	anti-skinning agent intermediate
Uses by professional workers	Coatings/printing inks (PC 9a, 18) Uses in laboratories (PC 21)	anti-skinning agent intermediate
Consumer uses	-	-
Article service life	-	-

Acetone oxime is used as anti-skinning agent for the preparation of coatings/printing inks. Acetone oxime is also used as intermediate for the manufacture of other substances/products. Intermediate use of oximes covers mainly manufacture of oxime silanes, which are applied as cross-linkers for silicon sealants. Consumer uses were not registered, but exposure of the general public is also expected to be possible via use of paints, printing inks and silicon sealants in non-industrial settings.

6 DATA SOURCES

ECHA dissemination site https://echa.europa.eu/de/substance-information/-/substanceinfo/100.103.524

In addition, original study reports, scientific literature as well as the Substance Evaluation Report, the CLH dossier and the RAC opinion of butanone oxime (Germany, 2014; Germany, 2017; RAC, 2018) served as information sources. Please see section 12. References for details.

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid, white	ECHA dissemination site [Feb, 2020]	-
Melting/freezing point	59.9°C (101.3 kPa)	ECHA dissemination site [Feb, 2020]	OECD 102
Boiling point	134 ± 1 °C (99.2 kPa)	ECHA dissemination site [Feb, 2020]	OECD 103
Relative density	1.06	ECHA dissemination site [Feb, 2020]	OECD 109
Vapour pressure	242 Pa (25°C)	ECHA dissemination site [Feb, 2020]	OECD 104
Surface tension	-	ECHA dissemination site [Feb, 2020]	-
Water solubility	327 g/l (20°C) 30.3 to 32.7% w/w at 20.0 ± 0.5 °C.	ECHA dissemination site [Feb, 2020]	OECD 105
Partition coefficient n-octanol/water	0.077 (22.7°C)	ECHA dissemination site [Feb, 2020]	OECD 107
Flash point	-	ECHA dissemination site [Feb, 2020]	Substance is a solid
Flammability	Highly flammable	ECHA dissemination site [Feb, 2020]	EU Method A.10
Explosive properties	-	ECHA dissemination site [Feb, 2020]	No chemical groups associated with explosive properties present in the molecule
Self-ignition temperature	-	ECHA dissemination site [Feb, 2020]	Waiving: melting point < 160°C
Oxidising properties	-	ECHA dissemination site [Feb, 2020]	The substance is incapable of reacting exothermically with combustible materials, on the basis of the chemical structure
Granulometry	-	ECHA dissemination site [Feb, 2020]	Waiving: substance not used in granular

Property	Value	Reference	Comment (e.g. measured or estimated)
			form
Stability in organic solvents and identity of relevant degradation products	-	ECHA dissemination site [Feb, 2020]	Not considered to be critical
Dissociation constant	pKa 12.42 (20°C)	ECHA dissemination site [Feb, 2020]	OECD 112
Viscosity	-	ECHA dissemination site [Feb, 2020]	Substance is a solid

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

For the toxicokinetics of acetone oxime several metabolism studies are available that are relevant for the read-across to butanone oxime (as summarized below in Table 7). In addition, the OECD Toolbox V.3.3 was used to gain additional information on metabolites.

Table 7: Summary table of metabolism studies

Method	Results	Remarks	Reference
In vitro liver microsomes study BALB/c mice, rat (SD), male 2 human liver samples Test substance: acetone oxime Test concentration: 20mM Incubation: 30 min at 37°C; Incubates contained microsomes equivalent to 0.25 g original liver per ml of incubate, acetone oxime (20 mM) and NADPH (3 mM) in buffer; Ion-pair HPLC and capillary GLC method and MS for metabolite analysis.	Results on metabolites: Propane 2-nitronate (P2-N) generated in the rodent microsomes were ~20 nmol/nmol cytochrome P 450 (same range was measured in human microsomes). If mice microsomes were selectively incubated after pre-treatment with inducers of the cytochrome P 450 isoenzymes some inducers enhanced the metabolite formation between 84% - 155% (likely isoenzymes from CYP2E1 and members of the CYP1A and CYP2B subfamily).	Study includes controls (heat-inactivated microsomes, no NADPH, replacement of air by nitrogen). Purity of acetone oxime was not reported. Klimisch 2 Amounts of P2-N or its neutral tautomer 2-NP were relatively small.	Kohl et al. (1992)
In vivo metabolism study Rat (SD), male Test substance: acetone oxime Test concentration: 3.36 mmol/kg in saline Administration i.p.	Acetone oxime and P2-N were excreted in urine in comparable concentrations (0.1 – 0.4 mM) during 76 hours. No information on excretion in faeces was reported.	Number of used animals is not reported. Purity of acetone oxime was not reported. No basic TK parameters were determined. Klimisch 3	Kohl et al. (1992)

Method	Results	Remarks	Reference
HPLC and GLC analysis In the *in vivo* metabolism study rats received either saline (control animals) or acetone oxime dissolved in the same vehicle; Animals were placed in metabolism cages; urine was collected for 76 hours.		Supportive information	
In vitro liver microsomes study B6C3F1 mice, rat (Wistar), male/female Human liver samples (4m/4f) Test substances: acetone oxime, butanone oxime (MEKO, purity 99.5%) Test concentration: 5 mM Incubation: 20 min at 37°C GC/MS analysis of selected metabolites	Metabolites of acetone oxime with liver microsomes include 2-nitropropane (2-NP; after tautomeric equilibration) and 2-nitro-1-propanol (in the range of 20 pmol/min/mg protein). Nitronate (P2-N) was formed at 167.5 pmol/min/mg (median), range 106.8 to 200.8 in 4 human liver microsomes (compared to 442.9 pmol/min/mg (median), range 174.5 to 892.9 in 8 human liver samples with butanone oxime). No sex differences in nitronate formation observed. Capacities for oxidation of liver microsomes to catalyse ketoxime oxidation was mice> humans >rats. Oxidation of acetone oxime was enhanced by P450 cytochrome that induced rates of nitronate formation. Though nitronate formation was lower for acetone oxime compared to MEKO species differences in the oxidation capacities were similar.	Study included controls, test systems and test items sufficiently described. Klimisch 2 Results showed that liver microsomes for all three species resulted in a slow oxidation of MEKO and acetone oxime to the corresponding nitronates. In addition to nitronate formation also direct oxidation to the corresponding nitroalcohol were shown. 2-nitropropane is in equilibrium with its tautomer propane-2-nitronic acid (in physiological media as the anion propane-2-nitronate)[1].	Völkel et al. (1999)
In vitro liver microsomes study SD rats (Sprague-Dawley rats treated with CYP 450 inducers) Test substance: acetone oxime and O-derivatives Test concentrations: 0.1-1 mM Incubation time 10 – 30 min HPLC/UV detection, ESR (Electron Spin Resonance) Measurements for hydroxyl radicals.	NADPH dependent metabolism of acetone oxime resulted in an accumulation of NO_2^- (around 5.8 µM from 1 mM Acetone oxime). The production of NO_2^- increased linearly with increasing concentrations of acetone oxime (possibly mediated by several CYP isoforms CYP1A, CYP2B and CYP2E1); nitric oxide (NO) was identified as an intermediate. A) Superoxide dismutase, catalase and the iron chelator desferrioxamine significantly inhibited the generation of NO_2^- (oxidative denitrification). B) iNOS (nitric oxide synthase) was not involved in the metabolism. C) Oxidative species generated were most likely hydroxyl radicals that preferentially interacts with the	Study includes controls, test systems and test items sufficiently described. Klimisch 2 The aim of the study was to characterize the oxidation of acetone oxime and to assess the ability of NOS (nitric oxide synthase, involved in NO generation from L-arginine) to catalyse the generation of nitric oxide from acetone oxime.	Caro et al. (2001)

[1]https://webwiser.nlm.nih.gov/substance?substanceId=93&identifier=2-Nitropropane&identifierType=name&menuItemId=44&catId=51

Method	Results	Remarks	Reference
	hydroxyl group rather than with its >C=function of acetone oxime earlier. D) Oxidative denitration of acetone oxime likely via formation of iminoxyl radicals.		
In vitro cell culture Test substances: Acetone oxime 98% purity, 2-NP and P2-N V79 (Chinese hamster) engineered cell lines for expression of individual sulfotransferases SULT 1A1 and SULT 1C1 from rat liver. Genotoxicity was determined by measuring the capacity of the test chemicals to induce DNA repair.	Acetone oxime did not activate rat sulfotransferase SULT 1A1 and SULT 1C1 in this study. 2-NP and P2-N were substrates for these enzymes and induced DNA repair synthesis. The authors suggested that the deoxygenation step in the proposed metabolic pathway of 2-NP occurs after the sulfonating step.	Study includes controls, test systems and test items sufficiently described. Klimisch 2	Andrae et al. (1999)
In vitro cell culture Test substances: Acetone oxime (purity 98%), P2-N and 2-NP V79 engineered cells expressing human sulfotransferases. Genotoxicity was determined by measuring the capacity of the test chemicals to induce DNA repair synthesis.	Acetone oxime did not induce DNA repair in any of the V79 cell lines and is thus not considered to be a substrate of the human phenol-sulphating and monoamine-sulphating phenol sulfotransferases and the hydroxysteroid sulfotransferase P2-N was activated by phenol sulfotransferases.	Study includes controls, test systems and test items sufficiently described. Klimisch 2	Kreis et al. (2000)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No information on skin metabolism was available. Therefore, the skin simulator of the OECD QSAR Toolbox V3.3.5[2] that mimics the metabolism of chemicals in the skin compartment was used. No skin metabolites were identified with the OECD tool.

The rat liver S9 metabolism simulator (OECD Toolbox) indicated oxidation of acetone oxime. The proposed structure is given in Figure 1.

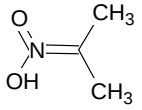


Figure 1: Predicted oxidation of acetone oxime to propane 2-nitronic acid (OECD Toolbox)

[2] https://www.qsartoolbox.org/

The hydrolysis products at acidic, basic and neutral pH, were predicted to be acetone and hydroxylamine (see Figure 2) according to the Hydrolysis Simulator of the OECD Toolbox. Taking findings for butanone oxime into account concerning pH dependency of hydrolysis, degradation is expected to be fast under acidic conditions (pH 4), to be significantly slower at neutral pH (experimental value for butanone oxime: 14% hydrolysis at was obtained after 4 days at pH 7 and 20°C[3]) and stable under basic conditions (pH 9). However, according to NTP (1999) hydrolysis of ketoximes *in vivo* is probably enzymatic and not simply a reaction of the oxime with water; for example, aqueous exposure solutions are quite stable. Also Bergström et al. (2008) stated that the hydrolysis might occur both enzymatically and non-enzymatically. Haas-Jobelius et al. (1991) showed no hydrolyses in incubation experiments with 200 µM acetone oxime for up to 90 min with primary hepatocytes and Chinese hamster cells (V79) as well as in control incubations without cells. However, the duration was too short at the assumed neutral pH value for hydrolysis.

Figure 2: Predicted hydrolytic metabolites (acetone and hydroxylamine) in aqueous solution (OECD Toolbox)

Metabolism studies from 2-butanone oxime indicated the existence of two metabolic pathways. The major pathway according to Germany (2014) is the hydrolysis of butanone oxime to butanone (MEK, methyl ethyl ketone). NTP (1999) also states that there is some evidence that the ketoxime is metabolized to the ketone and, presumably, hydroxylamine. Another major metabolite is CO_2. The second pathway is the oxidation of butanone oxime to butane–nitronate by microsomal monooxygenases, but this occurs at very low rates (without sex differences). Also the possibility of a third reductive pathway was indicated (Germany, 2014).

Conclusion**:**

No toxicokinetic study according to OECD guideline was available for acetone oxime. Physical chemical properties, QSAR estimates and information from an analogue oxime (butanone oxime) were considered. *In vitro* and *in vivo* metabolism studies showed that acetone oxime is converted in liver tissue of rats, mice and humans to P2-N (propane 2-nitronate) most likely by activation of cytochrome P450 enzymes. Amounts of P2-N and its neutral tautomer 2-nitropropane (2-NP) were reported to be small in *in vitro* and *in vivo* studies. *In vitro* experiments with mice and rats liver microsomes and human hepatocytes indicate that acetone oxime is metabolized to the corresponding nitronate at rates 50% of those observed with butane oxime oxidation (Völkel et al., 1999). However, 2-NP can also undergo cellular reduction to acetone oxime.

Acetone oxime was not a substrate of rat and human sulfotransferases but these enzymes were shown to play a role in the activation of P2-N. The formation of nitrite and the intermediate nitric oxide was experimentally proven in an *in vitro* rat liver microsome assay.

Based on metabolism studies with the analogue substance butanone oxime and the hydrolysis QSAR prediction for acetone oxime, another metabolic pathway could be the hydrolysis of acetone oxime. The hydrolysis may occur both enzymatically and non-enzymatically.

[3] https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/1

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not addressed in this CLH report.

10.2 Acute toxicity - dermal route

For acute dermal toxicity three experimental studies in rats and rabbits are available and summarized in the table below.

Table 8: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402 GLP Klimisch 1 Key study	CRL:(WI) rats, m/f 5/sex per dose	Test substance: acetone oxime (99.6%) Vehicle: water	Dose level: 2000 mg/kg applied to approximately 10% area of the total body surface Contact time: 24 hours Duration: 14 days	LD_{50} >2000 mg/kg No clinical signs were observed after the treatment with the test item or during the 14-day observation period. No effects on bw and no test item related findings of the macroscopic examination.	Unpublished study report (2012a)
Test guideline not stated No information concerning GLP Klimisch 3 Supportive study Strain not specified Method description und documentation incomplete Purity of test substance not reported	Rat, m/f 1/sex per dose	Test substance: acetone oxime Vehicle: water	Dose level: 100, 300, 1000 mg/kg Contact time: 24 hours Duration: 14 days	LD_{50} >1000 mg/kg Clinical signs: Lethargy in test animals at all dose groups; Body weight gain: dose dependent decrease in males during the observation period No macroscopic abnormalities in the post mortem examination.	Unpublished study report (1989a)
Similar to OECD Guideline 402 GLP Klimisch 2 Key study Purity of the test material was not	Rabbit (New Zealand White) 5/sex per dose (main test) 1/sex per dose (range finding	Test substance: acetone oxime	Dose levels range finding study include 1000 and 2000 mg/kg Dose levels main test: 0, 100, 500, 1000 mg/kg Contact time:	LD_{50} >1000 mg/kg <u>Main test:</u> Several animals in the high dose group (1000 mg/kg) were hypoactive, had fecal staining and exhibited a dark coloration to the eye (iris) at 24 hours and/or on day 2. Two animals showed poor food	Unpublished study report (1991b)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
specified. No details/methods of the neurological examination were reported.	study)		24 hours Vehicle: water Type of coverage: occlusive Study duration: 15 days	consumption. Low and mid dose group: single animals showed fecal staining. These effects were reversible at day 4. Haematology: dose-related methemoglobinemia on day 1 and anaemia on day 1 and 5. Effects on most of the haematology parameters in the high dose group in m and f. Statistical significant effects include <u>decreased:</u> haemoglobin, hematocrit and erythrocyte counts, <u>elevated:</u> reticulocyte count, mean corpuscular volume, mean corpuscular haemoglobin and mean leukocyte counts. Organ weights and body weights were unaffected. Neurological examination at day 1, 7 and 15 gave no unusual observation. Gross post-mortem observation revealed no treatment related abnormalities. Microscopic examination revealed myeloid and erythroid hypercellularity of the femoral bone marrow in 4 of 10 mid-dose animals and in all 10 high-dose animals. <u>Range-finding test:</u> 2/2 animals died at 2000 mg/kg and 1/2 at 1000 mg/kg.	

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Reliable LD_{50} values for classification of acetone oxime were derived from rats and rabbits, indicating that rabbits were more susceptible to effects caused by acetone oxime by the dermal route. In rats a LD_{50} >2000 mg/kg bw (limit test, GLP study according to OECD TG 402, Klimisch 1) was obtained; no treatment related clinical signs or effects were observed (unpublished study report, 2012a). In a second acute toxicity study in rats with one animal per sex per dose group no mortalities were observed up to the highest dose of 1000 mg/kg bw. However, study documentation and design qualifies this test as supporting information only (unpublished study report, 1989a). In a third acute dermal toxicity study performed under GLP and similar to OECD TG 402 in rabbits (unpublished study report, 1991b) the LD_{50} was determined to be >1000 mg/kg bw; based on the range finding study where a dose of 2000 mg/kg bw caused mortality in the two animals tested. The purity of the test item acetone oxime was not reported, the study was rated as Klimisch 2. In the main test clinical signs were evident in several animals in the high dose group at 1000 mg/kg bw (hypoactive, fecal staining, dark coloration of the eye) and in a few animals in the mid and low dose groups (fecal staining). Concerning the observed toxic effects, methemoglobinemia and anaemia were observed. Organ

weights and body weights were unaffected. Microscopic examination revealed myeloid and erythroid hypercellularity of the femoral bone marrow in 4 of 10 mid-dose animals and in all 10 high-dose animals (unpublished study report, 1991b).

10.2.2 Comparison with the CLP criteria

According to Table 3.1.1 of Regulation (EC) No. 1272/2008 a substance shall be classified as

- Acute Tox 4 (dermal) if the LC_{50}/ATE values are > 1000 and ≤ 2000 mg/kg bw
- Acute Tox 3 (dermal) if the LC_{50}/ATE values are > 200 and ≤ 1000 mg/kg bw

For the evaluation of acute dermal toxicity two GLP compliant, well reported guideline studies in two species are available. In rats the LD_{50} was > 2000 mg/kg bw (unpublished study report, 2012a), in rabbits the LD_{50} was > 1000 mg/kg bw in the main test, but below 2000 mg/kg bw based on the range-finding study (unpublished study report, 1991b). The third, less reliable study in rats supports the results with a LD_{50} of > 1000 mg/kg bw (unpublished study report, 1989a).

In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested (ECHA, 2017b). For the dermal route rats and rabbits are preferred for the evaluation of acute dermal toxicity. Different species sensitivity could be one plausible reason for the diverging of LD_{50} values/ranges.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the lowest LD_{50} value of > 1000 mg/kg bw but ≤ 2000 mg/kg bw in rabbits (unpublished study report, 1991b), a classification as Acute Tox. 4, H312 is proposed. An ATE value of 1100 mg/kg bw has to be assigned based on the conversion values from Table 3.1.2, Regulation (EC) No. 1272/2008.

10.3 Acute toxicity - inhalation route

Not addressed in this CLH report as no data is available.

10.4 Skin corrosion/irritation

Table 9: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr>
<td>Similar to OECD Guideline 404

GLP

Klimisch 2

key study

Longer exposure period than stated in the OECD guideline (4 hours)</td>
<td>6 rabbits (New Zealand White)

4 m/2 f</td>
<td>Test material: acetone oxime

Negative and positive (Sodium Lauryl Sulfate) controls reported</td>
<td>0.5 g of test item either applied as solid or moistened with 0.5 ml physiological saline solution was applied at the skin with a gauze patch. This patch was affixed to the application site.

Coverage: occlusive

Exposure period 24h</td>
<td>The effects were recorded in accordance with the DRAIZE scores.

Scores represent values averaged over days 1, 2, and 3:
<table>
<tr><th>Animal No.</th><th><u>Erythema score (moistened)</u></th><th><u>Edema score (moistened)</u></th></tr>
<tr><td>1</td><td>2</td><td>1</td></tr>
<tr><td>2</td><td>0</td><td>0</td></tr>
<tr><td>3</td><td>1.3</td><td>0.7</td></tr>
<tr><td>4</td><td>0</td><td>0</td></tr>
<tr><td>5</td><td>1.3</td><td>0.7</td></tr>
<tr><td>6</td><td>0.3</td><td>0</td></tr>
</table>
Slight irritating effects were fully reversible at day 5 in all 6 animals.</td>
<td>Unpublished study report (1990a)</td>
</tr>
</table>

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In a GLP compliant study similar to OECD TG 404 (rated Klimisch 2) six rabbits were exposed to 0.5 g acetone oxime/site for an exposure period (occlusive) of 24 hours to test possible skin irritation/corrosivity. In addition, rabbits were exposed to 0.5 g acetone oxime/site, moistened with physiological saline solution for 24 hours and scored according to Draize. The mean scores are presented in Table 9. Acetone oxime induced slight to well-defined erythema and slight edema on skin of some rabbits following a 24h-exposure period. All observed effects were fully reversible after 5 days (unpublished study report, 1990a). Also in the dermal acute toxicity studies summarized in Table 8 no local effects indicative of irritating or corrosive properties were reported.

10.4.2 Comparison with the CLP criteria

According to Table 3.2.2 of Regulation (EC) No. 1272/2008 a classification for irritation category 2 (in the case of six rabbits tested; see ECHA, 2017b) applies if:

(1) Mean score of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 4 of 6 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

In the available study performed under GLP and similar to OECD TG 404 (unpublished study report, 1990a) six rabbits were used and the mean individual scores from gradings at 24, 48 and 72h for erythema and edema ranged from 0 to 2 and 0 to 1, respectively. Skin reactions were not delayed. Effects were fully reversible after 5 days.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

According to the GLP study (unpublished study report, 1990a) no classification for skin irritation is proposed. The criteria for skin irritation Category 2 (at least 4 out of 6 rabbits have to show a mean score per animal of ≥ 2.3 to ≤ 4.0 for erythema/eschar or for oedema) is not met.

10.5 Serious eye damage/eye irritation

Table 10: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Similar to OECD Guideline 405 Klimisch 2 Key study GLP	3 female (f) and 3 male (m) rabbits (New Zealand White)	Test item: acetone oxime	0.1 g or 0.1 ml volume of the test substance was applied with no vehicle	Irreversible effects on the eye based on corneal damage that were not reversible within 21 days. Also conjunctival irritation and iridial changes or damage were observed. One hour after exposure all 6 animals exhibited conjunctival irritation (redness, chemosis, discharge and/or necrosis), 5 had minor iridial changes or damage, 5 had slight dulling of the	Unpublished study report (1990b)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
The effects were recorded in accordance with the DRAIZE scores.			Duration: 21 days	corneal surface and 1 had corneal opacity. Day 1: 6 animals exhibited Grade 3 discharge and Grade 2 conjunctival redness and chemosis, conjunctival necrosis, corneal opacity and/or corneal ulceration (absence of corneal epithelium). Day 3: Corneal opacity and iris score were max. 3 and 2 in one animal, respectively. Additional, during the observation period iris score were 1 in three animals and 0 in the remaining two. Corneal opacity score was 2 in one animal and 1 in three animals and 0 in the remaining one animal. Two animals also exhibited pannus on days 7 - 21 and days 10 – 21, respectively. Ocular irritation and effects were reversible except in two animals, one that continued to show corneal opacity ulceration and pannus at day 21, the other animal with persistent pannus from day 10 to study termination. Mean scores from 24, 48 and 72 hour observations for individual animals are listed in Table 11. Results of 3 animals with rinsed eyes after 24 hours (after application) are not shown. The severity of responses was generally comparable to that seen in unwashed eyes, with the exception that no pannus was observed.	

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

For the available *in vivo* study (unpublished study report, 1990b) six rabbits were exposed to 0.1 ml acetone oxime (or 0.1 g) in the eye. The study is well reported, performed under GLP and was rated Klimisch 2. Additionally three rabbits were dosed only for 20 seconds following washing of the eye for approximately 60 seconds. Assessment was made approximately 1 hour and on a daily base until day 4, day 7, 10, 14 and 21 post-treatment. The determined Draize scores for corneal opacity and iris scores were max. 3 and 2 in one animal at day 3, respectively. During the whole study period, iris score were 1 in three animals, 1.3 in one animal and 0 in the remaining two. Corneal opacity score were 2 in one animal and 1 to 1.3 in four animals and 0 in the remaining one animal during the study period. Mean individual scores from 24/48/72 hour observations are presented in Table 11.

Corneal ulceration with score 4 in 3/6 animals occurred at day 1, 1/6 at day 2. All animals, except one, recovered until day 21. Pannus was observed in two animals, these effects continued till study termination. Necrosis of the conjunctivae occurred in 6/6 animals at 24 hours till day 7 after exposure. Ocular irritation and effects were reversible except in two animals: one that continued to show corneal opacity, ulceration and pannus at day 21, the other animal with persistent pannus from day 10 to study termination.

Table 11: Individual mean Draize scores over 24/48/72h (unpublished study report, 1990b).

Mean scores over 24/48/72h	Animal #1	Animal #2	Animal #3	Animal #4	Animal #5	Animal #6
Chemosis (1)	2	2	1.7	2	1.7	1.3
Conjunctivae score (2)	2.3	2.6	2	2.6	2	2
Iris score (1)	1	1	1	1.3	0	0
Cornea opacity (2)	1.3(1)	1 (5, 4, 3)	1 (2)	2 (3, 4)	1 (2)	0

(1) fully reversible after 7 days (2) fully reversible within 14 or 21 days (3) not reversible after 21 days, (4) pannus (5) alopecia around eye

10.5.2 Comparison with the CLP criteria

According to Regulation (EC) No. 1272/2008, 3.3.2.6.1 substances classified in Category 1 (irreversible effects on the eye) based on animal data have to show observations listed in Table 12.

Table 12: Criteria for irreversible eye effects according to Table 3.3.1 of Regulation (EC) No. 1272/2008

Category 1:	A substance that produces: (a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or (b) in at least 2 of 3 tested animals, a positive response of: (i) corneal opacity ≥ 3 and/or (ii) iritis > 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

The regulation also stipulates: “These observations include animals with grade 4 cornea lesions and other severe reactions (e.g., destruction of cornea) observed at any time during the test, as well as persistent corneal opacity, discoloration of the cornea by a dye substance, adhesion, pannus, and interference with the function of the iris or other effects that impair sight. In this context, persistent lesions are considered those, which are not fully reversible within an observation period of normally 21 days. Hazard classification as Category 1 also contain substances fulfilling the criteria of corneal opacity ≥ 3 or iritis > 1,5 observed in at least 2 of 3 tested animals, because severe lesions like these usually do not reverse within a 21 days observation period.”

The actual test protocol with acetone oxime used 6 animals. For this study design the CLP guidance (ECHA, 2017b) indicates:

“In the case of 6 rabbits, the following applies:

(1) Classification for serious eye damage – Category 1 if:

(a) at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(b) at least 4 out of 6 rabbits show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis

(2) Classification for eye irritation – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of:

(a) ≥ 1 for corneal opacity and/or

(b) ≥ 1 for iritis and/or

(c) ≥ 2 conjunctival erythema (redness) and/or

(d) ≥ 2 conjunctival oedema (swelling) (chemosis) and which fully reverses within an observation period of normally 21 days."

Acetone oxime produced severe eye lesions in New Zealand rabbits (GLP study, unpublished study report, 1990b). The classification criteria for serious eye damage (b) corneal opacity ≥ 3 and/or iritis > 1.5 are not met because individual mean Draize scores over 24, 48 and 72 hours were below these mean scores in all tested animals. However, the scores for corneal ulceration were 4 in three animals at day 1 and in one animal at day 2. In addition to corneal ulceration also pannus was observed in two animals. Ocular irritation and effects were reversible except in two animals, one that continued to show corneal opacity, ulceration and pannus at day 21, the other animal with persistent pannus from day 10 to study termination. Necrosis of the conjunctivae was observed in 6/6 animals at 24 hours till day 7 after exposure.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on persistent severe eye lesions (pannus, corneal ulceration) observed in an animal study acetone oxime meets the criteria for classification and labelling as 'irreversible effects on the eye' Category 1, (Eye Dam. 1), H318.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

For the evaluation of this endpoint a guinea pig maximisation test (GPMT) (key study), a mouse ear swelling test (supporting study) and a Local Lymph Node Assay (LLNA) are available (see Table 13). No human data on the sensitising potential of acetone oxime are available.

Table 13: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Guinea Pig Maximisation Test (OECD Guideline 406) Klimisch 2 Key study GLP	guinea pig, Dunkin-Hartley, f N=15 (test group, pos. control) N=5 (neg. control)	Test substance: acetone oxime	Concentration: <u>Induction</u>: Intradermal: 5% in distilled water Topical induction: 100% (solid material, moistened with 0.9% saline). <u>Challenge</u> (epicutaneous, occlusive): 100% (solid material, moistened with 0.9% saline).	Sensitizing <u>Results</u>: 24h after challenge: 6/15 (40%) 48h after challenge: 5/15 (33%) <u>Neg. control</u>: 24h after challenge: 0/5 48h after challenge: 0/5 <u>Pos. control</u> (2,4-dinitrochlorobenzene): 24h after challenge: 15/15 48h after challenge: 15/15	Unpublished study report (1990c)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Mouse ear swelling test Klimisch 4 Non guideline test, limited documentation, no positive control	Mouse, Balb/c, f N=10 (test group) N=5 (neg control)	Test substance: acetone oxime	Induction: epicutaneous, open; 35% w/v (days 1, 2, 3, 4 and 7) Challenge: epicutaneous, open, 17.5% w/v (day 14, 21)	Not sensitizing Pretreatment of mice with FCA on day 0 No reaction in 10 dosed and 5 negative control animals	Unpublished study report (1989b)
LLNA (OECD Guideline 429) Klimisch 1 GLP Range finding study included	Mouse (CBA), female N= 4/group	Test substance: acetone oxime, 25µl purity: 99.6%, Vehicle: acetone/olive oil (AOO) (4:1)	acetone oxime (50%, 25%, 10% (w/w) in AOO on day 1, 2, 3 neg control: vehicle pos control: HCA (25% w/v)	Not sensitising Negative control (AOO): SI 1.0 Positive control (25 (w/v) % HCA in AOO): SI 10.7 Acetone oxime: 50 (w/v) % in AOO: SI 1.3 25 (w/v) % in AOO: SI 1.7 10 (w/v) % in AOO: SI 1.6	Unpublished study report (2013)

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

A GPMT (GLP study, according to OECD TG 406) with acetone oxime showed a clear response in 6/15 treated guinea pigs (equates to 40%) 24 hours after challenge (unpublished study report, 1990c). In this study 15 female guinea pigs received an intradermal injection of 5% acetone oxime in distilled water for induction. On day 7, a topical induction occluded patch was applied with acetone oxime (moistened with 0.9% saline). On day 21, animals were challenged with a topical occlusive patch moisten with 0.9% saline and acetone oxime. 24 and 48 hours after challenge 6/15 and 5/15 animals showed a positive response, respectively. Positive and negative control groups showed the expected results.

In a mouse ear swelling test (MEST) acetone oxime (35% w/v in milli-RO water) was applied to the skin of the abdomen of 10 mice on days 0 (with FCA intradermally injected), 1, 2, 3, 4 and 7 (induction). On day 14 a challenge and on day 21 a re-challenge was done with 17.5% w/v acetone oxime. Five animals were used as negative control. Ear thickness of test and control ears was measured at 0, 24 and 48 hours after application of the test substance. An animal was considered to be sensitized when an increase of ear thickness was measured after treatment of the ear greater than 20%. No positive reaction was seen in dosed and negative control animals. The MEST is no standard test method but it is a useful model for identifying strong contact sensitisers. To enhance the sensitivity (for moderate and weak sensitizer) animals shall be fed with a vitamin A-supplemented diet. No information on diet is given in the report. Beside this the number of tested animals is lower as recommended, the timeline for dosing (induction and challenge) different, the concentration of the challenge lower as recommended and the reporting is very rudimental. Therefore the test is not assignable for the evaluation of the skin sensitizing property of acetone oxime.

In 2012 a LLNA (according to GLP) was conducted according to OECD TG 429 (unpublished study report, 2013). 4 animals/group were exposed to vehicle acetone:olive oil (4:1) (neg. control), 25%

HCA (pos. control) or acetone oxime (50%, 25%, 10% (w/w) in AOO) on study days 1,2 and 3 (topical, dorsal surface of ear). Based on solubility in the vehicle the maximum concentration was 50%. The test substance showed no irritating property or systemic toxicity in a preliminary dermal toxicity test using 2 mice/dose. In the main study no signs of systemic toxicity or mortality were observed. Cell proliferation was investigated on day 6 via injection of ^{3}HTdR in the tail vein. The appearance of the lymph nodes was normal in treated groups and negative control group. The observed stimulation index values were 1.3, 1.7 and 1.6 at concentrations of 50, 25 and 10 (w/w) % acetone oxime, respectively. Using this method acetone oxime showed no sensitizing property.

10.7.2 Comparison with the CLP criteria

The criteria for the endpoint skin sensitisation are listed in Table 14.

Table 14: Hazard categories and sub-categories for skin sensitisers according to Table 3.4.2 and 3.4.4 of Regulation (EC) No. 1272/2008

Subcategory 1A	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered. For GPMT: ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose
Subcategory 1B	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered. For GPMT: ≥ 30 % to < 60 % responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose

Acetone oxime shows a clear positive result in a GPMT with 40% response after 5% intradermal induction. A LLNA and a non guideline MEST gave negative results.

These results are in line with results for the similar substance butanone oxime that showed also two positive GPMTs but a negative LLNA (RAC, 2018).

These conflicting results may be the result of basic differences between the available tests for sensitisation. In a LLNA the indicator for sensitisation is lymphocyte proliferation after topical application (induction) of the test substance. The GPMT is an adjuvant-type test in which the acquisition of sensitisation is potentiated by the use of Freund's Complete Adjuvant (FCA) and in which both intradermal and topical exposure are used during the induction phase. The variability of results due to the vehicle chosen for the LLNA is also known (ECHA, 2016). Therefore despite the negative result from the LLNA (and the non guideline MEST) the positive GPMT and the supporting evidence from the similar substance butanone oxime indicate a sensitizing potential.

In general oximes can readily be hydrolysed to the corresponding ketones or aldehydes, which are chemically reactive electrophilic compounds and can react with nucleophilic groups in macromolecules in the skin, thereby producing complete antigens and inducing contact allergy (Nilsson, 2005). This hydrolysis may occur both enzymatically and non-enzymatically (Bergström, 2008). As a second product of this reaction hydroxylamines will be released. Acetone oxime itself will be hydrolysed to acetone and hydroxylamine, a known sensitizer. Hydroxylamine has a harmonised classification (Index No 612-122-00-7 and 612-122-01-4) as Skin sens. 1, H317[4] according to Annex VI of Regulation (EC) No 1272/2008. The tendency to degradation by hydrolysis will be in the same order of magnitude like for the analogue butanone oxime (the rate of reaction increasing in acidic

[4] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/64606

conditions). For butanone oxime pH-dependent hydrolysis in water has been experimentally determined (see ECHA dissemination website[5] - hydrolysis, environmental fate) resulting in a half-live of <0.3 min at pH 4, >7d at pH 7 and no degradation at pH 9. The pH level of the skin is acidic, ranging from pH 4 to pH 7, with a natural level below pH 5 (Lambers, 2006).

10.7.3 Conclusion on classification and labelling for skin sensitisation

Acetone oxime shows clear evidence of skin sensitisation in guinea pigs with 40% responding animals at a 5% intradermal induction dose. This is supported by positive GPMT results with the similar substance butanone oxime and information on the hydrolysis product hydroxylamine. Classification as Skin Sens, sub-category 1B (H317) is proposed because a ≥ 30% responding at > 1% intradermal induction dose in a GPMT with acetone oxime was observed.

10.8 Germ cell mutagenicity

Acetone oxime has been evaluated in a battery of genotoxicity studies comprising of *in vitro* gene mutation assays in bacterial cells, *in vitro* gene mutation assays in mammalian cells, *in vitro* unscheduled DNA synthesis assays, *in vitro* comet assay as well as *in vivo* DNA and RNA adduct formation and the SMART assay. A summary of the standard information requirements including results and reliability scores is shown in Table 15.

No information concerning *in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study with acetone oxime are available. Instead information from read-across with analogues are depicted in Table 16. *In vivo* studies with the analogues are listed in

5 https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/7/5/2/?documentUUID=abf72f27-2330-4016-a942-e4c06b7df1ea

Table 17.

Table 16: Summary table of mutagenicity/genotoxicity tests *in vitro* (analogues butanone oxime and Wasox-MMAC2, Wasox-VMAC2)Table 15: Summary table of mutagenicity/genotoxicity tests *in vitro* (acetone oxime)

Test system / Study	**Concentration range or dose levels tested**	**Results**		**Reference/ Remarks**
In vitro gene mutation assay, bacterial reverse mutation test Similar to OECD 471 *S. typhimurium (strains TA 1535, TA 97, TA 98 and TA 100)* Test substance: acetone oxime Standard NTP study protocol, preincubation method	Main test - S9: 0, 100, 333, 1000, 3333, 10000 µg/plate. Main test + S9 (10% and 30% of male SD rat and Syrian hamster, respectively): 0, 100, 333, 1000, 3333, 10000 µg/plate. Negative and positive controls included	**+ S9** –	**- S9** –	NTP (2002) GLP Klimisch 2 Key study -maximum test concentration for soluble non-cytotoxic substances of 5 mg/plate exceeded -only 4 strains tested -no detailed study report was available, however NTP is regarded as reliable information source
In vitro gene mutation assay, bacterial reverse mutation test Test method according to Maron and Ames (1983). *S. typhimurium (strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100)* Test substance: acetone oxime Spot assay (=suspension method) and plate assay.	Plate assay: TA100 and *TA 98*: Test - S9/+S9: *TA 100* and *TA 98*: 0.25 – 2.5 µg/plate. Plate assay: *TA 100* and *TA 1535*: Test + S9: 2 – 8 mg/plate. Spot assay: *TA 1535*, *TA 1537*, *TA 1538*, *TA 98* and *TA 100*: Test +/- S9 Authors reported high volatility; No information on controls included	**+ S9** –	**- S9** –	Mirvish et al. (1998) No GLP Klimisch 3 Supportive study - strains slightly different compared to OECD 471 -results on purity not reported -no detailed study report, not all test concentrations were documented -maximum OECD recommended test concentration of 5 mg/plate exceeded for *TA 100* and *TA 1535*
In vitro gene mutation assay bacterial reverse mutation test *S. typhimurium (strains TA 2637, TA 98 and TA 100), E. coli WP2* uvrA/*pKM101* Test substance: acetone oxime preincubation method (37°C, 20 min)	-/+ S9 no concentrations nor controls reported/included	**+ S9** –	**- S9** –	Araki et al. (1986) No GLP Klimisch 3 Supportive study -no detailed study report was available -no information on purity, controls
Mammalian cell gene mutation assay (gene mutation) Former OECD 476, current OECD 490 Mouse lymphoma L5178Y cells Test substance: acetone	negative -Test + S9, 3h treatment (duplicate): 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL -Test –S9, 3h treatment: 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL -Test –S9, 24h treatment: 5000; 3750; 2500; 1250; 625	**+S9**	**- S9**	Unpublished study report (2012b) GLP Klimisch 2 Key study -cytotoxicity was determined by relative

Test system / Study	Concentration range or dose levels tested	Results		Reference/ Remarks
oxime, purity 99.6% 3 and 24-hour treatment with/without metabolic activation Phenotypic expression period 3 days	and 312.5 µg/mL: Acceptability criteria cloning efficiency for solvent and untreated control not met (<65%). Positive control – S9: 4-Nitroquinoline-N-oxide Positive control + S9: Cyclophosphamide solvent (DMSO) and untreated controls	–	–	survival, not by relative total growth (RTG) as recommended by the OECD guideline; thus relative cell growth during treatment and expression was not considered; -top dose selection not in line with new recommendations; -acceptability criteria for 3 assays met.
mammalian cell gene mutation assay (gene mutation) Similar to OECD 476 Chinese hamster lung fibroblasts (V79) Test substance: acetone oxime, purity: 98% Treatment period: 3 hours Concentration of 6-thioguanine: 11 µg/ml	0 (solvent), 0.23, 0.45, 0.5 mM acetone oxime (- S9) Solvent DMSO (1% v/v) Positive controls valid Top dose was chosen based on 20% RS (relative survival) Positive response with: Isopropyl hydroxylamine	**+ S9** No	**- S9** –	Haas-Jobelius et al. (1991) no GLP Klimisch 3 Supportive study Only 1 out of 5 acceptability criteria (selection of top dose) was sufficiently documented in the study. -spontaneous mutant frequency of the control not in the recommended range of $5x10^{-6}$ -no metabolic activation used -no OECD recommended reference substance used.

Table 16: Summary table of mutagenicity/genotoxicity tests *in vitro* (analogues butanone oxime and Wasox-MMAC2, Wasox-VMAC2)

Test system / Study	Concentration range or dose levels tested	Results		Reference/ Remarks
In vitro chromosome aberration test Similar to OECD Guideline 473 Chinese hamster ovary (CHO) cells Test item: butanone oxime, purity 99.5%	Dose; up to 5000 µg/L Up to 200 first-division metaphase cells were scored/dose No induction of chromosome aberration in cultured CHO cells with and without S9 activation	**+ S9** –	**- S9** –	NTP (1999) evaluated in Germany (2014) GLP assumed Read-across, supportive -up to 200 (instead of 300) metaphase cells were investigated and scored per concentration
In vitro chromosome aberration test, OECD Guideline 473 Primary human lymphocytes Test item: Wasox-MMAC2; Reaction mass of propan-2-one-O,O'-(methoxymethylsilandiyl)dioxime; propan-2-one-O-(dimethoxymethylsilyl)oxime; propan-2-one-O,O',O''-(methylsilantriyl)trioxime (CAS 797751-44-1) 3 and 20-hour treatment with/without metabolic activation	Test - 5% S9, 3h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Test - 5% S9, 20h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL: The highest test substance concentration was not analysed for chromosome aberrations, Mitotic Index (MI) of 7%, due to a very high cytotoxicity, which impeded scoring. At 1670 µg/L MI of 45% Test + 5% S9, 3h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Controls: Methylmethanesulfonate –S9, Cyclophosphamide +S9	**+ S9** –	**- S9** –	Unpublished study report (2005a) Klimisch 2 GLP Read-across, supportive -100 instead of 300 metaphases were investigated per concentration -the report did not state that also for the 3h incubation period 1.5 cell cycles occurred. -no information on by-products or impurities of the test item

Test system / Study	Concentration range or dose levels tested	Results		Reference/ Remarks
		+ S9	**- S9**	
In vitro chromosome aberration test, OECD Guideline 473 Primary human lymphocytes Test item: Wasox-VMAC2 UVCB, Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl]oxime (CAS 797751-33-0) 3 and 20-hour treatment with/without metabolic activation	Test - S9: 3h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Test -S9: 20h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL: The two highest test substance concentrations were not analysed (MI of 5% and 40%). The other doses caused test substance concentrations related numerical and structural chromosome aberrations (multiple chromatid breaks, fragments or interchanges). Test + 5% S9: 3h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Controls: Methylmethanesulfonate –S9, Cyclophosphamide +S9		3h: – 20h: + 3h: -	Unpublished study report (2005b) Klimisch 2 GLP Read-across, supportive -200 instead of 300 metaphases were investigated per concentration -the report did not state that also for the 3h incubation period 1.5 cell cycles occurred. -no information on by-products or impurities of the test item

Table 17: Summary table of mutagenicity/genotoxicity tests in mammalian cells or germ cells *in vivo* (analogues butanone oxime and Wasox-VMAC2)

Test system / Study	Concentration range or dose levels tested	Results	Reference/ Remarks
Chromosome aberration assay similar to EPA OPPTS 870.5385 (*In vivo* Mammalian Cytogenetic Tests: Bone Marrow Chromosomal Analysis) rat (Sprague-Dawley) male/female, 5/dose Test item: butanone oxime oral: gavage Vehicle: water	Dose levels 300, 600 and 1200 mg/kg bw Test results: toxicity: yes; vehicle controls valid, positive controls valid. In a chromosome aberration assay in male and female Sprague-Dawley rats no significant increase in chromosomal aberrations in the bone marrow was found after single oral doses by gavage of up to 1200 mg/kg bw butanone oxime (Germany, 2014).	Negative	Unpublished study report (1990d) Klimisch 2 Read-across Key study Original study not available; study evaluated by Germany (2014)
Mammalian Erythrocyte Micronucleus Test, OECD 474 Mouse, strain Crl:NMRI BR 5 m/f per dose; high dose and control 10 m/f Test item: Wasox-VMAC2, Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl] oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy (vinyl)silyl]oxime Sampling 24 and 48 hours after treatment.	Single dose of 1000, 1500, and 2000 mg/kg bw Vehicle: corn oil The dose volume was uniformly 10 mL per kg body mass. No cytotoxicity in the bone marrow was noted (PCE/NCE ratio not effected) at 2000 mg/kg bw (highest dose tested according to the guideline) Positive control: 40 mg/kg bw Cyclophosphamide	Negative	Unpublished study report (2007) GLP Klimisch 2 Read-across, supportive -no information on by-products and impurities

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Acetone oxime does not produce gene mutations in studies with prokaryotic cells *in vitro* (NTP, 2002; supported by Mirvish et al. 1998 and Araki et al. 1986), either in the presence or absence of a mammalian metabolic activation system. In GLP compliant gene mutations assays suitable to detect not only gene mutations, but also to some extent the induction of structural chromosomal mutations; acetone oxime produced negative results with and without metabolic activation (unpublished study report, 2012b, supported by Haas-Jobelius et al. 1991).

No adequate tests with acetone oxime for structural chromosome aberrations/clastogenicity were available. According to ECHA (2016) non-testing methods such as read-across approaches, may also provide information on the mutagenic potential of a substance. Therefore information from the analogue substance butanone oxime was considered (cf. Table 16).

Please see Annex I read-across justification for chemical identity, physico-chemical similarities, common metabolites and mammalian toxicity that allow butanone oxime to serve as a source substance in the read-across to acetone oxime.

In cytogenetic tests with butanone oxime and cultured Chinese Hamster Ovary (CHO) cells, no induction of sister chromatid exchange (SCE) was observed at concentrations up to cytotoxicity (500 µg/ml, -S9) or up to the assay limit (5000 µg/ml, +S9). No increase in chromosomal aberrations was observed in cultured CHO cells treated with up to 5000 µg/ml (+/-S9) butanone oxime according to Germany (2014) citing NTP (1999).

Moreover *in vitro* testing data of two additional substances were used in a weight of evidence approach (cf. Annex I read-across justification). In a GLP conform *in vitro* chromosome aberration test with human lymphocytes the vinyl substituted silane (Wasox-VMAC2) was positive without metabolic activation and 20 hour treatment. The substance induces numerical and structural chromosome aberrations consistent with multiple chromatid breaks, fragments or interchanges in this test system (unpublished study report, 2005b). The methyl substituted silane (Wasox-MMAC2) did not induce structural chromosome aberrations under the same test conditions (unpublished study report, 2005a). Whether this difference is associated with the vinyl/methyl silane portion of Wasox-VMAC2 is unclear. To further investigate the mutagenicity an *in vivo* mammalian erythrocyte micronucleus test was performed to detect the possible formation of micronuclei, induced by Wasox-VMAC2 (as a result of chromosomal damage or of damage to the mitotic apparatus of mice). The test substance did not produce relevant increases of the numbers of micronuclei in polychromatic erythrocytes in animals of either sex of the test species at a single dose of 1000, 1500 or 2000 mg/kg bw after 24 and 48 hours oral administration. However, no cytotoxicity in the bone marrow was shown (no proof that Wasox-VMAC2/metabolites reached the target tissue). As the result of this study was negative it can be assumed that none of the two hydrolysis products including acetone oxime were positive in this system. While relatively high doses were tested, there is no proof of systemic bone marrow exposure.

An *in vivo* study with the analogue butanone oxime (cf. Table 17) tested in a chromosome aberration assay in male and female Sprague-Dawley rats did not significantly increase chromosomal aberrations in the bone marrow after single oral doses by gavage of up to 1200 mg/kg bw (unpublished study report, 1990d; Germany, 2014).

Indicator tests (detecting putative DNA lesions):

Additional literature studies exploring further the genotoxic potential of acetone oxime are available. Tests for genotoxicity include assays which provide an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as DNA strand breaks, unscheduled DNA synthesis, sister chromatid exchange or DNA adduct formation (according to ECHA, 2016).

Acetone oxime caused no induction of DNA repair in V79 cell lines (V79-MZ, V79-rHSTa, V79-rHST20, V79-rPST-IV and V79-rST1C1 cells) indicating that it is not a substrate for rat sulfotransferases SULT1A1 and SULT1C1[6]. The treatment period was 5 hour at three concentrations of 1, 3 and 10 mM without metabolic activation (Andrae et al. 1999; cf. Table 7). The principle of the study followed partly the deleted OECD test guideline 482 and can be used as supportive study. Also with a similar test design exploring the human sulfotransferases as activation system for acetone oxime Kreis et al. (2000) showed that the compound did not induce DNA repair in V79 cell lines capable of expressing individual human sulfotransferases (V79 -HP-PST, V79 -hM-PST, V79 –hHPST). The treatment period was 5 hour at a concentration up to 10 mM without metabolic activation (Kreis et al. 2000; cf. Table 7).

In ovine seminal vesicle (OSV) cells that lack cytochrome P450 enzymes but express phenol sulfotransferase acetone oxime did not induce DNA repair or any detectable DNA modification (DX1, 8-aminodGuo, 8–oxodGuo) in OSV cells or in cultured rat hepatocytes according to Kreis et al. (1998). Also Haas-Jobelius et al. (1991) found no induction of DNA repair (test protocol partly in line with OECD 482, full reporting lacking) in primary rat hepatocytes and V79 cells including positive and negative controls.

An *in vitro* alkaline comet assay using cultured human lymphoblastoid cell line TK6 and acetone oxime concentrations from 625 to 10000 µM including solvent (DMSO) and positive (etoposide)

[6] Sulfotransferases are suggested to play a role in the activation of 2-NP and are also discussed for the mediation of butanone oxime to a carcinogenic agent (please see also Annex I).

controls the test compound did not induce a statistically significant increase in tail intensity (unpublished study report, 2016; no GLP). The comet assay can detect single and double strand breaks in eukaryotic cells, however an international test guideline only exists for *in vivo*, therefore this information is considered as supportive study.

Hussain et al (1990) investigated DNA and RNA adduct formation of acetone oxime and 2-nitropropane (2-NP) in vivo in male SD and male F344 rats by gavage and i.p. administration, respectively. The used vehicle was 4:1 water-Emulphor 620. Liver DNA and RNA were analysed after 6 hour following administration. Detection of 8-hydroxyguanine (8-oxoguanine, 8-OH-G) levels in liver DNA and RNA were increased compared to control and showed a similar pattern in both species. No significant strain differences were observed for 8-OH-G. Quantitative results in SD rats showed that 8-hydroxy-2'-deoxyguanosine (8-OH-dG) formation after i.p. administration of 2-NP was in the same range as measured after oral administration of acetone oxime. However acetone oxime was administered at an approximately 3 times higher dose. The amount of detected DNA modifications (8-OH-dG, unknown modified deoxynucleoside DX1) was approximately half compared to 2-NP and around one third of the RNA modifications (8-OH-GR, RX1, RX2) caused by 2-NP in F344 rats after i.p. administration. In summary the main DNA and RNA modifications were 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanine; the DNA modification was around 3 times elevated compared to control and in RNA 6 times higher. The unknown RNA modification RX2/GR was 7 to 9 times higher compared to control after i.p. or oral administration of acetone oxime in F344 and SD rats, respectively.

Another study, Guo et al. (1990) supported the previous findings and demonstrated that observed DNA and RNA modifications were markedly higher (factor 1.6/4.9 for DNA/RNA) in male SD rats than female rats after 18 hours acetone oxime i.p. administration. Some of the reported modifications were not or only at very low levels detected in females indicating less oxidative damage to nucleic acids in the livers of female SD rats. Also an increase in 8-OH-dG and 8-OH-G by a factor of 2.4 and 5.8 for DNA and RNA for males compared to controls and other DX1 DNA base modification were reported. Adduct formation also increased with time (results after 6 hours not presented). Kidney DNA and RNA modifications were not detectable (Guo et al. 1990).

In liver RNA from butanone oxime exposed rats, a dose, sex and time-dependent formation of 8-aminoguanosine and 8-oxoguanosine, but no DNA adduct formation was observed. Concentrations of this modification in RNA were approximately 5 times higher in male rats as compared to female rats exposed to identical 8-aminoguanosine concentrations (Germany, 2014).

Ryskova et. al (1997) investigated the genotoxic potential of acetone oxime up to 5000 µM in the SMART assay (Somatic Mutation and Recombination Test) in *Drosophila melanogaster* using non-transgenic strains and strains expressing the bacterial lacZ gene or the human HGST (human glutathione S-transferase). Genotoxicity was measured by determination of the frequency of homozygous mutant spots per wing. Acetone oxime showed a weak dose related increase in the induction of wing spots in non-transgenic and transgenic flies compared to N-Nitroso-N-methylurea. However, significant increases in number of spots per wing occurred in non-transgenic flies already at 0.5 µM (frequency of spots 0.73) compared to control (frequency 0.3) and increased in a dose dependant manner to 1.08 (frequency) at 5000 µM. Depending on the copies of the HGST gene wing spots were significantly reduced with three copies compared to control (Ryskova et al. 1997).

10.8.2 Comparison with the CLP criteria

According to CLP Regulation for the purpose of classification for mutagenicity, substances are allocated to one of two categories (Table 18).

Table 18: Hazard categories for germ cell mutagens according to Table 3.5.1 of Regulation (EC) No. 1272/2008

Category 1	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans Substances known to induce heritable mutations in the germ cells of humans
Subcategory 1A	The classification in Cat. 1A is based on positive evidence form human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Subcategory 1B	The classification in Category 1B is based on: — positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or — positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
Category 2	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: — somatic cell mutagenicity tests in vivo, in mammals; or — other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

No epidemiological studies are available for acetone oxime and thus no classification in Cat. 1A is warranted. There is no *in vivo* heritable germ cell mutagenicity test available or evidence that the substance has potential to cause mutations to germ cells, which would qualify for classification into Cat. 1B.

There is sufficient information available for the evaluation of germ cell mutagenicity of acetone oxime. Read-across from butanone oxime und evidence from oxime silanes were also considered for evaluation of this endpoint.

Acetone oxime did not induce reverse mutations in *Salmonella typhimurium* strains (NTP, 2002, key study, supported by Araki et al. 1986 and Mirvish et al. 1998). In mammalian *in vitro* systems acetone oxime did not cause gene mutations in mouse lymphoma cells or Chinese hamster fibroblasts (unpublished study report, 2012b and supportive study Haas-Jobelius et al., 1991).

Information from analogue substances (butanone oxime and methyl or/and vinyl substituted oxime silanes) cover clastogenicity/aneuploidy. Butanone oxime did not induce chromosome aberrations in cultured Chinese hamster ovary cells (NTP, 1999). The only evidence from standard *in vitro* tests that acetone oxime can cause chromosome aberration is based on a positive result with methyl/vinyl substituted oxime silane as test substance (that hydrolysis rapidly to acetone oxime). The substance induced structural and numerical damage to chromatids/chromosomes in peripheral human lymphocytes (unpublished study report, 2005b). Weather this difference is associated with the methyl/vinyl silane portion of Wasox-VMAC2 is unclear because the result with the methyl substituted analogue (same study design and laboratory) was negative (unpublished study report, 2005a). Furthermore, in another *in vivo* experiment, the SMART assay with Drosophila (Ryskova et.

al. 1997), acetone oxime showed a dose related increase in wing spots in non-transgenic and transgenic flies indicative for genotoxicity. However, this assay has no international harmonisation or validation.

In vivo studies were carried out with read-across substances. The studies have been conducted according to guidelines and GLP criteria. In an *in vivo* chromosome aberration assay with butanone oxime in rats no significant increase of chromosomal aberrations in the bone marrow occurred (unpublished study report, 1990d). RAC concluded that butanone oxime has been shown to be non-genotoxic and no classification for germ cell mutagenicity is warranted (RAC, 2018). An *in vivo* mammalian erythrocyte micronucleus test with Wasox-VMAC2 did not produce relevant increases of the numbers of micronuclei in polychromatic erythrocytes, however there was no proof of reaching the target tissues up to a dose of 2000 mg/kg bw (unpublished study report, 2007).

Also supportive studies concerning indirect evidence of DNA damage, but not direct evidence of mutagenicity, showed that acetone oxime did not induce DNA strand breaks in an *in vitro* Comet assay (unpublished study report, 2016) or induce DNA damage in unscheduled DNA synthesis in *in vitro* studies (Andrae et al. 1999, Kreis et al. 2020). However two non-guideline investigations (Hussain et al. 1990 and Guo et al. 1990) indicate that acetone oxime can cause DNA and RNA adduct formation (main modification 8-hydroxyguanine) in liver of F344 and SD rats after i.p. or oral administration indicating oxidative stress.

The standard *in vitro* tests indicate that acetone oxime does not induce gene mutation or chromosomal aberration. The outcome is supported by GLP and guideline conform *in vivo* mutagenicity studies with analogue substances. On the other hand there are positive effects in *in vivo* studies indicating that the substance induces DNA and RNA modifications in liver of exposed rats. However, it is taken into account that adduct formation does not necessarily lead to mutation.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on available studies with acetone oxime and the read-across to butanone oxime no classification for germ cell mutagenicity according to the CLP Regulation (EC) 1272/2008 is proposed.

10.9 Carcinogenicity

No guidelines carcinogenicity studies with acetone oxime are available, however, concern regarding the carcinogenic potential of acetone oxime comes from the structurally related substance butanone oxime. For the carcinogenic potential of acetone oxime the following information sources were considered:

Read-across from the structural analogue substance butanone oxime

According to ECHA (2016) carcinogens may be identified also by extrapolation from structurally similar substances (read-across). The justification for the read-across approach to butanone oxime is described in detail in Annex I. Butanone oxime and acetone oxime are structurally similar, the toxicity pattern of the two compounds is to some extent comparable and both possess an endpoint specific structural alert for carcinogenicity according to QSAR estimations. The carcinogenic potential of butanone oxime has been studied in two combined chronic toxicity/carcinogenicity studies and in two animal species (cf. Table 19).

Table 19: Summary table of animal studies on carcinogenicity (butanone oxime)

Study/Method	Results	Remarks/ Reference
similar to OECD TG 453 rat (F344) male/female 50/sex/group Test substance: butanone oxime	Positive: Liver tumours: Lowest exposure level causing a significant increase 75 ppm (270 mg/m^3) (RAC, 2018) males: liver carcinomas 0/50, 0/51, 1/51, 12/51; statistically significant at 374 ppm	Newton et al. (2001) Germany (2014) and RAC (2018) Klimisch 2 Key study

Study/Method	Results	Remarks/ Reference
Purity: 99.9% 0, 15, 75, 374 ppm equivalent to 54, 270, 1346 mg/m^3, Inhalation: vapour, 6h/d, 5d/week Duration: 26 months interim sacrifice at 3, 12 and 18 months;	males: liver adenomas 0/50, 2/51, 5/51, 18/51; statistically significant at 75 and 374 ppm males: fibroadenomas in mammary gland 2/50, 2/50, 4/50, 9/50; statistically significant at 374 ppm At study termination testes weight was elevated by 82% compared to control without microscopic findings females: liver adenomas 0/50, 0/50, 2/50, 4/51; not statistically significant females: fibroadenomas in mammary gland 10/50, 7/50, 9/50, 17/50; not statistically significant	
similar to OECD TG 453 CD-1 mice, male/female 50/sex/group Test substance: butanone oxime Purity: 99.9% 0, 15, 76, 374 ppm Inhalation: vapour, 6h/d, 5d/week Duration 18 months, interim sacrifice at 12 months;	Positive: Liver tumours; 374 ppm (1346 mg/m^3) for liver carcinoma Carcinomas in males at 374 ppm (1346 mg/m^3); and adenomas in all test groups ≥ 15 ppm (≥ 54 mg/m^3); decrease in latency for liver carcinomas at 374 ppm males: liver carcinomas 2/50, 2/50, 1/50, 10/50; statistically significant at 374 ppm males: liver adenomas 4/50, 11/50, 10/50, 11/50, not statistically significant, but within historical control range females liver adenomas 0/50, 0/50, 1/50, 3/50; not statistically significant	Newton et al. (2001) Germany (2017) and RAC (2018) Klimisch 2 Key study

Histopathological findings from the 90-day study with acetone oxime

Table 20: Summary table of 90-d repeated dose toxicity study (acetone oxime)

Study/Method	Results	Remarks/ Reference
equivalent or similar to OECD Guideline 408 Rat (Sprague-Dawley) male/female 25/sex/dose 5/sex/dose and 10/sex/dose were sacrificed after 45 days and 90 days, respectively.	NOAEL: 10 mg/kg bw/d (based on effects on the hematopoetic system) Histopathology liver: Clear liver cell foci were present in almost all high dose animals with slight to severe/high grading at day 90. Also basophilic cell foci in all animals at the high dose were observed ranging from minimal to moderate. Foci of cellular alteration in the liver were already observed at 50 and 250 mg/kg bw/d at	Unpublished study report (1991c) Klimisch 2 Key study GLP Dosing volume was not adjusted to the same volume for the different dose levels

Study/Method	Results	Remarks/ Reference
Test material: Acetone oxime Dose levels 0, 10, 50, 250 mg/kg bw/d Administration route: gavage Vehicle: water Study duration: 90 days followed by a 30 days recovery period	the interim sacrifice at day 45. The clear cell foci, composed of hepatocytes with clear finely granular cytoplasm, varied considerable in size and sometimes coalesced to form large areas of alternation. Basophilic cell foci consisted of more discrete alterations which were composed of hepatocytes with round central nuclei with prominent nuclear chromatin and basophilic staining cytoplasm. Cellular atypia, increased mitoses or compression were absent. Slight to moderate cytoplasmic vacuolization (characterized by intracytoplasmic accumulation of clear vacuoles resembling lipid) and slight bile duct proliferation was observed in males at the high dose level. The proliferating bile ducts were often in close association with macrophages containing hemosiderin-like pigment in the portal areas of the liver.	

Supportive evidence from non-guideline studies/investigations

Further experimental evidence for carcinogenicity is provided in Table 21.

Table 21: Summary table of animal studies on carcinogenicity (acetone oxime)

Study/Method	Results	Remarks/ Reference
Carcinogenicity study, non guideline Rat (MRC-wistar) male/female 15/16 m/f Test material: Acetone oxime oral: drinking water, 5 days/ week Dose level 1000 mg/L water, total dose/rat: 7 g/male rat, 6.2 g/ female rat Study duration: 18 months	LOAEL (carcinogenicity): ≤1000 ppm Incidence of liver tumours in male rats was 80% (12/15) at week 93 (statistically different to 0% in the control); in females 17% (3/16) incidence by week 111). Tumours were characterised as hepatocellular adenomas mostly 1-4 cm in diameter; composed of circumscribed masses of cells, having abundant cytoplasm and small, round nuclei; In 1 male focal malignant degeneration was described. 2 males had in addition haemangiomas.	Mirvish et al. (1982) No GLP Klimisch 3 Supportive study Purity: not stated Control group of 23/20 m/f rats were started 8 months apart because this group served also as controls for another trial. Limited study documentation Average daily doses for male and female were 25.4 mg/kg and 24.6 mg/kg bw/d, respectively (Carcinogenic Potency Database[7])
Rat liver foci model male MRC-Wistar and Wistar rats; up to 10 animals/strain Test material: acetone oxime	Significantly higher frequency of hyperplastic liver nodules (HLN) compared to control. Authors suggested that acetone oxime may be a liver promotor.	Mirvish et al. (1988) No GLP Klimisch 3 Supportive study

[7] https://toxnet.nlm.nih.gov/cpdb/chempages/ACETOXIME.html

Study/Method	Results	Remarks/ Reference
1000 ppm in drinking water single diethylnitrosamine (DEN) i.p. treatment (200 mg/kg bw) 2 weeks after DEN: test substance administration for 8 weeks 3 weeks after DEN: partial hepatectomy		Purity: not stated

QSAR information

The QSAR prediction from the QSAR Toolbox V3.3.5 indicated for this endpoint specific structural alert Category "Oncologic primary classification C-Nitroso and Oxime Type" for acetone oxime. However, no supporting mechanistic chemistry is available in this profiler. The profiler was developed by the Laboratory of Mathematical Chemistry (LMC) solely to mimic the structural classes of known/potential carcinogens covered in version 7.0 of the United States Environmental Protection Agency's (US EPA) OncoLogic Cancer Expert System for predicting carcinogenic potential.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Several information sources were used to evaluate the carcinogenicity of acetone oxime:

Read-across from the structural analogue substance butanone oxime

The combined chronic toxicity/carcinogenicity studies in rats and mice (similar to OECD TG 453) have demonstrated that butanone oxime causes liver tumours (adenomas and carcinomas) in both species at all tested exposure concentrations (cf. Table 19). Statistically significant increases in incidence were observed at 270 and 1346 mg/m^3 for liver adenomas in male rats and at 1346 mg/m^3 for liver carcinomas in male rats and male mice. An increased incidence of liver adenomas occurred also in female rats and mice at 270 and 1346 mg/m^3, but was not statistically significant. A dose-response relationship for tumour induction in the liver of rats and mice was observed in both sexes. The incidence of fibroadenomas in the mammary gland was also significantly increased in male rats at 1346 mg/m^3 (Germany, 2014).

RAC (2018) evaluated these studies in detail and concluded: "The long-term inhalation to vapours of butanone oxime led to a carcinogenic effect in both rats and mice. There were statistically significant increases in benign and malignant tumours in the livers of male rats and in malignant liver tumours in male mice exposed to butanone oxime. No such tumours were seen in control rats and the tumour rates in the control mice were low. There were also increases in hepatocellular adenoma in female rats and mice exposed to high levels of butanone oxime, relative to the concurrent controls, but these findings were not statistically significant. There were no increased levels of malignant liver tumours seen in female rats or mice.

There were no clear differences in the non-neoplastic findings in the livers of these animals to explain why males might have been more sensitive than females. In the absence of a clear mechanistic explanation for the increased liver tumours, both the findings in rats and mice are considered of relevance for human hazard assessment.

Additionally, an increased frequency of mammary gland fibroadenoma was observed in male rats exposed to the highest level of butanone oxime. No laboratory historical control data were provided for this benign lesion, but the frequency seen was substantially higher than that reported in the open literature. It is difficult to account for this finding. In females, there was a slight increase compared to controls in the frequency of these tumours, but this was not statistically significant and well within the control range described in the literature. There were no non-neoplastic changes in the mammary glands of rats exposed to butanone oxime that might explain how these tumours arose and no treatment-related effects were noted in the available reproductive studies. Overall, it is possible that

butanone oxime is carcinogenic to the mammary gland of male rats, but considerable uncertainty remains both about this finding and its relevance to humans" (RAC, 2018).

Histopathological findings from the 90-day study

Hepatocellular changes were more severe in acetone oxime treated male rats than in female rats in the 90-day study (unpublished study report, 1991c) indicating more pronounced effects in males, also for acetone oxime. Foci of cellular alteration are common in rodent studies with a duration greater than twelve months and may be seen in short duration toxicity studies following exposure to certain chemicals (Thoolen et al. 2010). Clear liver cell foci were present in almost all high dose animals with slight to sever grading, also minimal clear cell foci were detected in the mid dose group after 90 days (cf. Table 20). The used rat strain was Sprague-Dawley that does not have a high incidence rate for this lesion compared to F344 rats. Also basophilic cell foci were detected in all animals in the highest dose group. The observed foci of cellular alteration were more frequently observed in the males (also in the high dose group) compared to females. Dose dependant onset of foci of cellular alteration was already observed at day 45 (unpublished study report, 1991c).

Clear cell foci of cellular alteration have been designated to play a precursor role in the process of hepatocarcinogenesis as they represent a localized proliferation of hepatocytes that are phenotypically different from the surrounding liver. Thoolen et al. (2012) claimed that small cell changes (small liver cell dysplasia) in humans and basophilic cell foci in the rat showed common histomorphological characteristics, which might be indicative of a mutual presumptive role in the process of hepatocarcinogenesis. In conclusion these focal cellular alterations occur spontaneously in aged rats but are also considered as precursor lesions to hepatocarcinogenesis (Thoolen et al. 2012). It is understood that foci of cellular alteration can be found as non-neoplastic endstage lesions and not all foci can be related to carcinogens (Thoolen et al. 2010).

However the early onset and the high incidence of clear and basophilic cell foci found in the 90-day study with acetone oxime indicates that these lesions are tumour pre stages which further adds to the evidence that acetone oxime may cause liver tumours in rats.

Supportive evidence from non-guideline studies/investigations

Mirvish et al. (1982) investigated the carcinogenic potential of acetone oxime according to a non-guideline non-GLP compliant study. Acetone oxime was administered to male and female MRC Wistar rats at a dose of 1000 mg/L drinking water during 18 months. For males only, the liver tumour incidence of 80% was significantly higher compared to control. All these tumours had benign histologic criteria despite occasional differences in nuclear size, except in one male rat in which focal malignant degeneration was noted. Three rats (including 2 males) had liver haemangiomas in addition to the adenomas. Though the study has major deficiencies the finding concerning the carcinogenic property of acetone oxime cannot be neglected. From this study Gold et al. (1989) calculated a TD_{50} of 12.1 mg/kg bw/day (male rat). The general approach is described in the open source paper, however no detailed calculations are provided for the 492 substances included in Gold et al. (1989).

According to ECHA (2016) short and medium term bioassay data like the rat liver foci model, while less validated and standardised, can be used as supportive information. Mirvish et al. (1988) investigated acetone oxime in a HLN assay in Wistar and MRC-Wistar rats and found a significantly higher frequency of hyperplastic liver nodules compared to control.

QSAR information

The QSAR prediction from the QSAR Toolbox V3.3.5 gave the endpoint specific structural alert Category: Oncologic primary classification C-Nitroso and Oxime Type for acetone oxime.

10.9.2 Comparison with the CLP criteria

According to 3.6.2.2.2. of Regulation (EC) No. 1272/2008 the classification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories as listed in Table 22.

Table 22: Hazard categories for carcinogens according to Table 3.6.1 of Regulation (EC) No. 1272/2008

Category 1	Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Subcategory 1A	Category 1A (known to have carcinogenic potential for humans, classification is largely based on human evidence)
Subcategory 1B	Category 1B (presumed to have carcinogenic potential for humans, classification is largely based on animal evidence)
	The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: — human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or — animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
Category 2	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

To assess the carcinogenicity of acetone oxime no guideline and GLP compliant carcinogenicity study was available. Classification of a substance as a carcinogen is based on consideration of the strength of the evidence of available data for classification with considerations of all other relevant information (weight of evidence) being taken into account as appropriate (ECHA, 2016). In absence of robust and reliable experimental carcinogenicity information for acetone oxime results from the analogue substance butanone oxime are considered. According to a RAC opinion (2018) butanone oxime has been harmonized classified as Carc Cat. 1B; H350 (May cause cancer) with the general concentration limit of 0.1%.

Supportive experimental evidence from administration of acetone oxime to laboratory animals strengthens the concern that also acetone oxime is a liver carcinogen. In a 90-day repeated dose study in SD rats an early dose dependant onset of liver lesions consistent with foci of cellular alteration (clear cell foci, basophilic cell foci) were observed (unpublished study report, 1991c). These lesions were more abundant in male animals. In a 18 month chronic study hepatocellular adenomas in male MRC-wistar rats were induced after administration via drinking water (Mirvish et al., 1982). In a HLN assay in rats a significantly higher frequency of hyperplastic liver nodules compared to control were detected (Mirvish et al., 1988). Also a QSAR prediction gave a structural alert for carcinogenicity. The mode of action for carcinogenicity for acetone oxime is not established; however, based on the available mechanistic investigations and toxicokinetic information it can be assumed that metabolic activation to reactive intermediates and radical formation might play a role.

For butanone oxime RAC (2018) stated for the MoA that it is unlikely that blood toxicity was a factor in the hepatocarcinogenicity of butanone oxime and limited evidence to suggest a MoA that involved cytotoxicity. No other specific mechanism of action has been identified and thus the tumours observed are relevant to humans (RAC, 2018).

10.9.3 Conclusion on classification and labelling for carcinogenicity

In a weight of evidence approach several lines indicate that acetone oxime has a carcinogenic potential relevant for humans. Based on the read-across to butanone oxime and animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity acetone oxime is proposed to be classified as presumed human carcinogen, category 1B; H350 (May cause cancer) and the general concentration limit of 0.1% should apply. This conclusion is further justified by limited animal experiments with acetone oxime and QSAR information.

10.9.4 Specific concentration limit (SCL)

The non-guideline study available for acetone oxime (Mirvish et al., 1982), which only tested a single dose, which induced 80% tumor response in male rats is not adequate to derive a T_{25} value for acetone oxime. As the TD_{50} value mentioned by Gold et al. (1989) is not adequately described in this study, no T_{25} can be derived for acetone oxime itself. It seems not adequate to derive a T_{25} from the read across substance butanone oxime. It is however noted that the T_{25} value calculated by RAC (2018) for butanone oxime allocates this substance to the medium potency group and no SCLs were decided for butanone oxime. No SCLs are indicated for acetone oxime.

10.10 Reproductive toxicity

Not addressed in this CLH report.

10.11 Specific target organ toxicity-single exposure

In acute oral and dermal toxicity studies on acetone oxime, transient and reversible neurological effects were detected. The results of experimental studies regarding narcotic effects of acetone oxime and of the analogue substance butanone oxime are summarised in Table 23.

Table 23: Summary table of animal studies on STOT SE

Study/Method	Results	Remarks/Reference
Acute oral toxicity study, similar to OECD 401 Sprague-Dawley CD rats 5/sex/group Test substance: acetone oxime Dose levels: 0, 300, 1000 and 3000 mg/kg bw Vehicle: distilled water Oral: gavage Controls: distilled water Duration: 14 days Neurological examinations were performed on Days 0, 1, 7 and 14.	LD_{50} >3000 mg/kg 3000 mg/kg: ataxia immediately after dosing on day 1 (at 4 h) in 3 animals and hypoactivity up to 4 days after dosing in several animals; decreased food consumption; no abnormal reflexes or other indications of neurologic impairment; 1 male died at day 2; 1000 mg/kg: ataxia and hypoactivity 2 h after dosing in one animal and decreased food consumption in another animal. Dose related reduced body weights (bw) and bw gains (reversible from day 7 in the low dose group, only). Dose related methemoglobinemia (day 1, 300 and 3000 mg/kg dosed satellite group) and anemia. Gross post-mortem examination: increased significant absolute and relative spleen weights (males and females); microscopic examination revealed increased extramedullary haematopoiesis and pigments in reticuloendothelial cells compared to controls (generally without a clear dose relationship).	Unpublished study report (1991a) GLP Klimisch 2 Key study No appendices were submitted by the registrant; therefore the purity of acetone oxime is not specified. In a range finding study 2/2 animals died at 5000 mg/kg. Neurological examination revealed ataxia immediately after dosing that can be interpreted as signs of transient narcosis.
Test guideline not stated, dermal acute toxicity study Rat 1/m/f per dose Test substance: acetone oxime Dose level: 100, 300, 1000 mg/kg Contact time: 24 h Vehicle: water Duration: 14 days	LD_{50} >1000 mg/kg Clinical signs: Lethargy in test animals at all dose groups; Body weight gain: dose dependent decrease in males during the observation period No macroscopic abnormalities in the post mortem examination.	Unpublished study report (1989a) Strain not specified Method description und documentation incomplete Purity of test substance not reported No information concerning GLP Klimisch 3 Supportive study
Similar to OECD Guideline 402 Rabbit (New Zealand White) 5/sex/dose Test substance: acetone oxime	LD_{50} >1000 mg/kg Several animals in the high dose group (1000 mg/kg) were hypoactive, had fecal staining and exhibited a dark coloration to the eye (iris) at 24 hours	Unpublished study report (1991b) GLP Klimisch 2 Key study

Study/Method	Results	Remarks/Reference
Dose levels range finding study include 1000 and 2000 mg/kg Dose levels main test: 0, 100, 500, 1000 mg/kg Contact time: 24 h Vehicle: water Type of coverage: occlusive Study duration: 15 days	and/or on day 2. Two animals showed poor food consumption. Low and mid dose group: single animals showed fecal staining. This effect was reversible at day 4. Haematology: dose-related methemoglobinemia on day 1 and anaemia on day 1 and 5. Effects on most of the haematology parameters in the high dose group. Organ weights and body weights were unaffected. Neurological examination at day 1, 7 and 15 gave no unusual observation. In the range-finding test 2/2 animals died at 2000 mg/kg and 1/2 at 1000 mg/kg.	Purity of the test material was not specified. No details/methods of the neurological examination were reported. Study details/results of the range finding study including cause of the mortality are lacking.
Acute neurotoxicity study Rat m/f Test substance: butanone oxime Doses level: 0, 100, 300 and 900 mg/kg bw/d	LD_{50}, rat m/f>900 mg/kg bw At 900 mg/kg: no mortality; decreased activity 30-60 min after exposure LOEL 300 mg/kg bw: based on transient neurobehavioral effects (impaired gait, disturbed aerial righting reflex, reversible within 24h); suggested a transient narcoleptic response	Schulze and Derelanko (1993) Cited in Germany (2017) GLP
Acute inhalation toxicity study similar to OECD TG 403 Rat, F344, male/female, 5/sex/group Test substance: butanone oxime purity: > 98% vapour, 4 h; Concentrations tested: 0, 0.19, 1.45, 4.83 mg/L	LOAECrat f = 4.83 mg/L based on observation of narcotic effects During exposure strong temporary narcotic effect in both sexes	TL2 (1984), unpublished study report cited in Germany (2017) GLP
Developmental toxicity study according to OECD TG 414 Rabbit, New Zealand White; 18 f/dose Test substance: butanone oxime purity: > 99% Oral, gavage Doses level: 0, 8, 14, 24, 40 mg/kg bw/d Duration: GD6-18	Preliminary study (dose range-finding study): ≥ 40 mg/kg bw/d: clinical signs: laboured breathing, decreased activity, few or no faeces Main study: 40 mg/kg bw/d: clinical signs: decreased activity, wobbly gait, no faeces, ↓:bw, food consumption; $LOAEL_f$= 40 mg/kg bw/d based on neurobehavioral effects	TL19 (1990), unpublished study report cited in Germany (2017) Derelanko et al. (2003) GLP

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

After oral and dermal administration, ataxia, hypoactivity and/or lethargy were reported at higher dose levels in two species (rats and rabbits) in acute toxicity studies (unpublished study report, 1991a; unpublished study report, 1991b; supported by unpublished study report, 1989a).

In the available GLP conform study similar to OECD TG 401 (unpublished study report, 1991a) a group of five male and five female rats was treated with acetone oxime at a dose of 300, 1000 and 3000 mg/kg. The purity of the test substance was not specified in the provided documentation. Effects on the haematological system were investigated in a satellite group at 300 and 3000 mg/kg. Dose related reduced body weights (bw) and bw gains were observed in all dose groups, the effect was reversible from day 7 in the low dose group, only. Dose related methemoglobinemia and anemia were reported. In a range finding study 2/2 animals died at 5000 mg/kg. In the main test one animal died at the highest dose of 3000 mg/kg. The LD_{50} was found to be greater than 3000 mg/kg bw. While no details on the neurological examinations were available ataxia was reported as treatment related effect after oral administration of 3000 mg/kg. Ataxia and hypoactivity occurred also 2 hours after dosing in one animal at 1000 mg/kg in the same study. No neurological or clinical signs were observed at a low dose of 300 mg/kg (unpublished study report, 1991a).

In the acute dermal toxicity study similar to OECD TG 402 with rabbits the neurological examination at day 1, 7 and 15 gave no unusual observation at 100, 500 and 1000 mg/kg. Hypoactivity in the high dose group of 1000 mg/kg was observed in addition to other clinical signs (unpublished study report, 1991b). Therefore, it is unclear whether this observation was indicative of a temporary narcotic effect or signs of general toxicity due to impending health.

The third study which is less reliable based on only 2 rats tested per dose group and limited documentation reported lethargy of the animals in all dose groups at 100, 300, 1000 mg/kg after dermal exposure (unpublished study report, 1989a).

No other specific studies that address neurotoxicity were available. Derelanko and Rusch (2008) stated in their publication on structure/toxicity relationships of oxime silanes that narcosis has been found consistently with low molecular oximes such as acetone oxime, however, data on acetone oxime were unpublished according to the authors (Derelanko and Rusch, 2008).

The ECHA guidance states that a substance that has not been tested for specific target organ toxicity may, where appropriate, be classified on the basis of data from a validated structure activity relationship and expert judgement-based extrapolation from a structural analogue that has previously been classified together with substantial support from consideration of other important factors such as formation of common significant metabolites (ECHA, 2015).

The analogue substance butanone oxime met the classification for specific target organ toxicity after single exposure based on its narcotic effects in rats and rabbits after acute oral, inhalation and dermal exposure; STOT SE 3, H336 (May cause drowsiness or dizziness) (RAC, 2018). Please see Annex I for read across justification.

Neurotoxicity following acute and subchronic exposure to butanone oxime was studied in rats including a Functional Observational Battery, assessment of motor activity, and neuropathology evaluations (Schulze and Derelanko, 1993). Oral single doses of ≥ 300 mg/kg bw butanone oxime administered by gavage were found to produce transient and reversible changes in neurobehavioral function (changes in gait and aerial righting reflex) consistent with CNS depression, but no evidence of cumulative neurotoxicity was detected (Schulze and Derelanko, 1993; Germany, 2017). After subchronic exposure transient treatment-related changes in ease of cage removal, ease of handling, and in posture, gait, and aerial righting were observed at 400 mg/kg/day (Schulze and Derelanko, 1993), however in rabbits (dams) effects occurred at much lower dose levels at ≥ 40 mg/kg bw/d (cf. Table 23) (Derelanko et al. 2003). In the acute inhalation toxicity study a strong but transient narcotic effect occurred in both sexes at 4.83 mg/L/4h during the exposure (TL2, 1984; Germany, 2017). In addition also after dermal exposure, narcotic effects were observed at a dose of 18 mg/kg bw (Germany, 2017).

10.11.2 Comparison with the CLP criteria

According to Regulation (EC) No. 1272/2008 for the purpose of classification for specific target organ toxicity – single exposure, substances are allocated to one of three categories (cf. Table 24). No guidance values are provided for category 3 substances since this classification is primarily based on human data and, if available, animal data. The later shall be included in the weight of evidence evaluation according to the regulation.

Table 24: Hazard categories for specific target organ toxicity-single exposure and criteria for narcotic effects according to Table 3.8.1 and 3.8.2.2.2 of Regulation (EC) No. 1272/2008

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure. Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: (a) reliable and good quality evidence from human cases or epidemiological studies; or (b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/ concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) in order to help in classification. In exceptional cases, human evidence can also be used to place a substance in Category 2 (see 3.8.2.1.6).
Category 3	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.
	The criteria for classifying substances as Category 3 for narcotic effects are: (a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness; (b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

No human data with acetone oxime for this endpoint are available. Animal data indicate ataxia after oral administration in rats at a high dose (unpublished study report 1991a). Hypoactivity and lethargy were also described in rabbits and rats (unpublished study report, 1991b, 1989a), the finding in rabbits may be compromised by general systemic toxicity.

No investigations concerning the mode of action are available. While the evidence from the animal data is not fully comprehensive the chemical structure of low molecular oximes are indicative of narcotic effects.

The analogue substance butanone oxime met the classification for STOT SE 3, H336 (May cause drowsiness or dizziness) according to RAC (2018). The structural similarities to acetone oxime including the common functional oxime group may justify the consideration of such a classification for acetone oxime as well. The mechanism is not available or known for acetone oxime. Though the available data for acetone oxime on this endpoint is limited and effect levels for narcosis might be higher compared to butanone oxime there is concern that acetone oxime can elicit transient narcotic effects as evidenced by decreased activity, ataxia or lethargy in laboratory animals after single exposure. According to the ECHA guidance if a study shows clear evidence for narcotic effects at any dose level then this could support classification with Category 3 (ECHA, 2017b).

10.11.3 Conclusion on classification and labelling for STOT SE

Based on the available information there is sufficient evidence that acetone oxime meets the criteria for classification as STOT SE 3, H336 (May cause drowsiness or dizziness) according to Regulation (EC) No. 1272/2008.

10.12 Specific target organ toxicity-repeated exposure

For repeated dose toxicity results from an experimental study with acetone oxime in rats were available.

Table 25: Summary table of animal studies on STOT RE

Study/Method	Results	Remarks/Reference
Repeated dose 90-day oral toxicity in rodents, equivalent or similar to OECD Guideline 408 Rat (Sprague-Dawley) male/female 25/sex/dose 5/sex/dose and 10/sex/dose were sacrificed after 45 days and 90 days, respectively. Test material: acetone oxime Dose levels 0, 10, 50, 250 mg/kg bw/d Administration route: gavage Vehicle: water Study duration: 90 days followed by a 30 day recovery period	NOAEL: 10 mg/kg bw/d Effects indicative of anemia: =10 mg/kg bw/d (f, only at day 45): blood: statistically significant: ↓ haemoglobin (-9%), haematocrit and RBC ≥ 50 mg/kg bw/d (m/f) at 45 and 90 days: blood: elevated methaemoglobin, regenerative anaemia, compensatory reticulocytosis, erythrocytic morphology consistent with polychromia and occasional Howell-Jolly bodies; Spleen (90 d): ↑↑ absolute and relative weight (high dose not reversible, 30%-50% still increased compared to control after recovery) ≥ 50 mg/kg bw/d (m only) at 45 and 90 days: reversible thrombocytosis, ↓ cholesterol (30% compared to control), ↓ total protein and albumin = 250 mg/kg bw/d (m/f) at 45 and 90 days: liver: ↑ absolute and/or relative weights (in males not reversible >10%); males: ↑ relative heart weight and ↑ ALP at 90 d = 250 mg/kg bw/d (m/f) at 45 and 90 days: reversible leucocytosis, ↑ bilirubin, ↑A/G ratio (f), ↓ ALT (f). Extramedullary hematopoiesis in the liver and	Unpublished study report (1991c) Klimisch 2 Key study GLP Urine analysis lacking Dosing volume was not adjusted to the same volume for the dose levels No functional observations

Study/Method	Results	Remarks/Reference
	spleen (m/f) with increasing severity from 50 to 250 mg/kg and from 45 to 90 days.	

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a GLP compliant 90-day study (unpublished study report, 1991c) in rats at 10, 50 and 250 mg/kg bw/day, dose-related statistically significant methemoglobinemia, anemia and erythrocyte morphology changes were observed in mid- and high-dose animals. Therefore, it can be concluded that acetone oxime causes damage to mature erythrocytes in the peripheral blood, resulting in alterations in the measured erythrocyte-related parameters at the haematological examination. As a compensatory reaction reticulocytosis, hypercellularity in the bone marrow and haematopoiesis in the spleen and liver were observed with an increase in incidence and severity with time and dose. Urine was not sampled or analysed.

Haemoglobin was slightly but not statistically significantly decreased at 90-day study termination in male and female rats, in contrast to the 45 days finding: At this sampling point haemoglobin values were 10.1% and 13% statistically significantly decreased in males and females at 50 mg/kg bw/day, respectively (cf. Table 26).

Treatment-related changes of the liver and spleen were consistent with elevated liver weights in high dose animals and spleen weights in the mid- and high-dose group.

Differences in mean absolute and relative spleen weights were dose related and generally statistically significant. Weights at 50 mg/kg were approximately twice those of control animals, while spleen weights for high dose animals were approximately 3 to 4 times increased compared to control. At termination of the recovery period, spleen weights at 50 mg/kg were comparable to control values but for the high dose animal’s weights remained 30% to 50% higher than control values (cf. Table 26, unpublished study report, 1991c).

The histopathological changes in the liver and spleen were increased in a dose-related manner and were not reversible following the 30-day recovery period. Hepatocellular changes were more severe in treated male rats than in female rats and specific evidence of hepatotoxicity was observed only in treated males. Histopathology of the liver revealed clear cell foci, extramedullary hematopoiesis and pigmentation (suggested hemosiderin accumulation in the Kupffer cells lining the hepatic sinusoids and phagocytic macrophages in the periportal areas) in the mid- and high-dose groups.

Alterations in the spleen included dose dependant increases in extramedullary hematopoiesis, pigmentation and congestion of the red pulp. Capsular fibrosis was observed in one male and one female at 250 mg/kg (high dose group) (unpublished study report, 1991c).

Table 26: Selected hematology parameters, body weight, liver and spleen weight (unpublished study report, 1991c)

Dose/time	Methemo-globin (%)	Hemoglobin (g/dl)	Hematocrit (%)	Erythrocyte count (RBC) (mil/µl)	Reticulocyte count (%RBC)	Platelet count (100 T/µl)	Body weight (g)	Liver weight (organ/bw*100)	Spleen weight (organ/bw*1000)
45 d, n=5, male									
vehicle	0.4	16.9	44	7.27	0.3	11.33	418	3.19	1.81
10 mg/kg	0.7	16.5	44	7.23	0.7	12.12	421	3.25	1.93
50 mg/kg	2.9	15.2	40	6.03	2.2	15.35	414	3.47	3.87
250 mg/kg	6.9	15	35	4.66	34.4	15.35	431	4.33	7.75
45 d, n=5, female									
vehicle	0.8	17.3	46	7.19	0.2	10.99	226	3.25	2.14
10 mg/kg	0.7	15.7	42	6.65	0.8	12.13	249	3.13	2.37
50 mg/kg	1.8	15	39	5.71	2.4	13.79	234	3.14	3.56
250 mg/kg	5.5	14	34	4.51	38.6	13.76	228	3.74	8.37
90 d, n=10, male									
vehicle	0.8	15.2	45	7.64	0.2	11.55	498	3.09	2.09
10 mg/kg	1.1	15.2	45	7.64	0.6	12.42	528	2.98	1.78
50 mg/kg	3.2	15	44	6.8	1.6	14.09	501	2	3.11
250 mg/kg	8	14.6	38	5.28	13.7	15.01	480	3.59	7.55
90 d, n=10, female									
vehicle	0.6	15.3	45	7.25	1.6	12.05	273	2.88	2.66
10 mg/kg	0.9	15.9	48	7.51	0.6	10.91	279	2.79	2.66
50 mg/kg	2.7	14.2	42	6.25	1.8	11.20	274	2.76	3.66
250 mg/kg	7.3	14.9	39	5.45	12.8	13.82	271	3.38	6.90
Recovery, n=10, male									
vehicle	0.5	16.2	45	7.93	1.2	13.01	574	2.84	1.54

Dose/time	Methemo-globin (%)	Hemoglobin (g/dl)	Hematocrit (%)	Erythrocyte count (RBC) (mil/µl)	Reticulocyte count (%RBC)	Platelet count (100 T/µl)	Body weight (g)	Liver weight (organ/bw*100)	Spleen weight (organ/bw*1000)
10 mg/kg	0.4	16.6	46	8.2	0.3	13.71	580	2.87	1.54
50 mg/kg	0.5	16.8	47	7.9	0.3	12.88	564	2.93	1.54
250 mg/kg	0.5	18.3	51	7.94	0.6	12.17	553	3.31	2.3
Recovery, n=10, female									
vehicle	0.4	16.5	47	7.66	1	13.01	294	2.81	1.73
10 mg/kg	0.5	16.2	46	7.52	0.3	13.71	293	2.83	1.78
50 mg/kg	0.6	17.4	48	7.81	0.2	12.88	289	2.73	1.79
250 mg/kg	0.6	17.9	50	7.6	0.4	12.17	281	3.04	2.39

	Statistically significant p ≤0.05
	Statistically significant p ≤0.01

10.12.2 Comparison with the CLP criteria

According to Regulation (EC) No. 1272/2008 for the purpose of classification for repeated dose toxicity, substances are allocated to one of two categories:

Table 27: Hazard categories for specific target organ toxicity-repeated exposure according to Table 3.9.1 of Regulation (EC) No. 1272/2008

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: — reliable and good quality evidence from human cases or epidemiological studies; or — observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in Category 2.

Guidance values to assist in Category 1 (Table 3.9.2) and Category 2 (Table 3.9.3) are provided in the Regulation.

A repeated dose study in rats indicated that haemolytic anemia was the main toxic effect corresponding to decreased red blood cell parameters and increased breakdown products of haemoglobin, increased pigmentation (indicated to consist of deposits of iron, hemosiderin) and extramedullary hematopoiesis in spleen and liver (unpublished study report, 1991c). Haemolytic anemia is consistently found with lower molecular weight ketoximes according to Derelanko and Rusch (2008).

In rat effects on the blood were observed in sub-chronic oral toxicity studies at doses of ≥ 50 mg/kg bw/d. Compared to the interim study results the anemia (haemoglobin decrease of 10.1% and 13% in males and females at 50 mg/kg bw/d at day 45, respectively) is compensated leading to a slight, but not statistically significant decrease in haemoglobin values at 90-days (cf. Table 26). Other erythrocyte parameters like methaemoglobin, erythrocyte count, mean corpuscular volume and mean corpuscular haemoglobin in males and females as well as reticulocyte and platelet counts in males were statistically significant different compared to control at 50 mg/kg and at 90-day study termination. At 50 mg/kg the interim sacrifice (day 45) and at study termination (day 90) a statistically increase of absolute and relative spleen weights for female occurred; at study termination also in males at the highest dose (in females this effect was not reversible at the highest dose). The corresponding histopathological effects in the spleen were extramedullary hematopoiesis, pigmentation and congestion of the red pulp. Capsular fibrosis was observed in two animals in the high-dose group.

The observed methemoglobinemia does not result in lethality but exposure to acetone oxime results in signs of damage to the erythrocytes, haemolysis and anaemia. According to ECHA (2017b) the formation of methaemoglobin shall be classified accordingly either in STOT SE or STOT RE and is warranted if any consistent and significant adverse changes in haematology is observed at the guidance values for category 2: oral (rat): $10 < C \leq 100$ mg/kg bw/d (Annex I, Part 3, Table 3.9.3 of Regulation (EC) No. 1272/2008). The assessment shall take into consideration not only significant

changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs according to CLP Annex I, 3.9.1.4 (ECHA, 2017b).

The observed effects include the reduction of haemoglobin ≥10% at 45 days in both sexes, reduction of RBC of 14% in females and of 11% in males at study termination at 50 mg/kg bw/d, together with significant increase of haemosiderosis in the spleen accompanied by increased organ weight. Also slight to moderate haemosiderin accumulation in the liver as well as minimal to slight extramedullary hematopoiesis was observed at 50 mg/kg bw/d.

This combination of effects on the hematopoietic system and associated organs demonstrate an adverse effect after repeated exposure (90 days) to 50 mg/kg bw/day. In addition RAC concluded on the analogue substance butanone oxime that the substance should be classified as STOT RE 2; H373 (May cause damage to the blood system through prolonged or repeated exposure).

10.12.3 Conclusion on classification and labelling for STOT RE

Based on adverse effects on the hematopoietic system and associated organs, acetone oxime should be classified for target organ toxicity through repeated exposure (STOT RE 2, H373 (blood system)) according to Regulation (EC) No. 1272/2008.

10.13 Aspiration hazard

Not evaluated in this CLH report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not addressed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not addressed in this dossier.

13 ADDITIONAL LABELLING

Not relevant

14 REFERENCES

Andrae et al (1999). Activation of propane 2-nitronate to a genotoxicant in V79-derived cell lines engineered for the expression of rat hepatic sulfotransferases. Mutation Research, 439, 191–197.

Araki et al (1986). Mutagenicities of oxime compounds in S. typhimurium TA98, TA100, TA2637 and E. coli WP2uvrA/pKM101. Mutation Research, 164, 263.

Bergström M.A, Luthman K, Nilsson JLG, Ann-Therese Karlberg A-T (2005). An α,β -unsaturated oxime identified as a strong contact allergen Indications of antigen formation via several pathways. Food and Chemical Toxicology 43, 1627–1636.

Bergström MA, Andersson SI, Broo K, Luthman K, Karlberg A-T (2008). Oximes: Metabolic Activation and Structure-Allergenic Activity Relationships. J. Med. Chem. 2008, 51, 2541–2550.

Blunder M, Hurkes N, Spirk S, List M, Pietschnig R (2011). Silanetriols as in vitro inhibitors for AChE. Bioorg Med Chem Lett. 2011 January 01; 21(1): 363–365.

Caro AA, Cederbaum AI, Stoyanovsky DA (2001). Oxidation of the ketoxime acetoxime to nitric oxide by oxygen radical-generating systems.Nitric Oxide. 2001 Aug;5(4):413-24.

Chemspider (2017). ChemSpider database, http://www.chemspider.com/Chemical-Structure.4481809.html, 2017-07-17

Derelanko, Michael J., Rinehart, W. E. and Rodwell, Dean E. (2003). Developmental toxicity studies of methyl ethyl ketoxime (MEKO) in rats and rabbits. Drug and Chemical Toxicology 26(3): 147-168, 2003.

Derelanko MJ & Rusch, (2008). Oxime silanes: structure/toxicity relationships, Drug and Chemical Toxicology, 31:97-114, 2008.

ECHA (2008). Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals, May 2008. https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9, 2017-03-20

ECHA (2016) Guidance on information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance. Draft Version 5.0 https://echa.europa.eu/documents/10162/13643/ir_csa_r7a_r7-3_caracal_draft_en.pdf, 2017-03-20

ECHA (2017a) Read-Across Assessment Framework (RAAF), ISBN: 978-92-9495-758-0https://echa.europa.eu/documents/10162/13628/raaf_en.pdf, 2017-03-20

ECHA (2017b) Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017, https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5, 2020-03-20

Germany (2014). Substance Evaluation Report Butanone oxime (MEKO), BAuA, June 2014. https://echa.europa.eu/documents/10162/2f03502e-c4f8-5498-b713-cbae02c9d5a1, 2020-08-20

Germany (2017). Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 Substance Name: Butanone BAuA, May 2017. https://echa.europa.eu/documents/10162/973f4ac8-f234-6de5-dd48-0d5d6ac202c9 , 2020-08-20

Gold LS, Slone TH, Bernstein L (1989). Summary of Carcinogenic Potency and Positivity for 492 Rodent Carcinogens in the Carcinogenic Potency Database. Environ Health Perspect. 79 (0), 259-272. https://ehp.niehs.nih.gov/doi/pdf/10.1289/ehp.8979259

Guo et al (1990). Sex and organ differences in oxidative DNA and RNA damage due to treatment of Sprague-Dawley rats with acetoxime or 2-nitropropane. Carcinogenesis, 11(9), 1659-1662

Hass-Jobelius et al (1991). Nitroreduction is not involved in the genotoxicity of 2-nitropropane in cultured mammalian cells. Mutagenesis, 6(1), 87-91.

Lambers H, Piessens S, Bloem A, Pronk H, Finkel P (2006). Natural skin surface pH is on average below 5, which is beneficial for its resident flora. Int J Cosmet Sci. 2006 Oct;28(5):359-70.

Hussain NS, Conaway CC, Guo N, Asaad W, Fiala ES (1990). Oxidative DNA and RNA damage in rat liver due to acetoxime: similarity to effects of 2-nitropropane. Carcinogenesis vol.11, nº 6, pp. 1013-1016.

Kohl et al (1992). Acetoxime is metabolised by human and rodent hepatic cytochrome P450 enzymes to the genotoxicant and carcinogen propane 2-nitronate. Carcinogenesis, 1397), 1091-1094.

Kreis P, Degen G, Andrae U (1998). Sulfotransferase-mediated genotoxicity of propane 2-nitronate in cultured ovine seminal vesicle cells. Mutation Research 413 (1998) 69-81.

Kreis P, Brandner S, Coughtrie M, Pabel U, Meinl W, Glatt H, Andrae U (2000). Human phenol sulfotransferases hP-PST and hM-PST activate propane 2-nitronate to a genotoxicant. Carcinogenesis vol.21 no.2 pp. 205-299, 2000.

Maron and Ames (1983). Revised methods for the Salmonella mutagenicity test. Mutation Research, 113, 173-215.

Mirvish SS, Salmasi S, Runge RG. (1982). CARCINOGENICITY TEST OF ACETOXIME IN MRC-WISTAR RATS. J. NATL. CANCER INST. 69:961-962, 1982. Testing laboratory: University of Nebrasca Medical Center, USA.

Mirvish SS et al (1988). Induction of hyperplastic liver nodules in Wistar and MRC-Wistar rats by phenobarbital and the liver carcinogens acetoxime, 1-nitroso-5,6-dihydrouracil and 3-nitroso-2-oxazolidinone.Cancer Letters, 41(2), 211-216 (1988).

Newton et al. (2001). A chronic inhalation toxicity/oncogenicity study of methylethylketoxime in rats and mice. Inhalation Toxicology, Volume 2001; 13(12): 1093–1116.

NTP (1990). 2-Nitropropane, Report on Carcinogens, Fourteenth Edition https://ntp.niehs.nih.gov/ntp/roc/content/profiles/nitropropane.pdf, 2017-03-20.

NTP (1999). Technical Report on the Toxicity Studies of Methyl Ethyl Ketoxime; Administered in Drinking Water to F344/N Rats and B6C3F Mice, National Toxicology Program Toxicity Report Series Number 51; NIH Publication 99-3947; U.S. Department of Health and Human Services; Public Health Service http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox051.pdf, 2017-03-20.

NTP (2002). The *Salmonella* mutagenicity test with Acetoxime. NTP database. NTP Report no A86757. https://ntp.niehs.nih.gov/testing/status/agents/ts-m010029.html, 2017-03-20.

OECD (2006). SIDS INITIAL ASSESSMENT PROFILE: Acetaldehyde oxime, SIAM 23, 17-20 October 2006 US/ICCA, http://webnet.oecd.org/hpv/ui/SIDS_Details.aspx?id=481ab9a1-4066-499a-b350-3230a976f212 and http://webnet.oecd.org/hpv/ui/SIDS_Details.aspx?id=481ab9a1-4066-499a-b350-3230a976f212 2017-03-19.

OECD (2009). SIDS INITIAL ASSESSMENT PROFILE Oximino Silanes Category SIAM 28, 15-17 April 2009 US/ICCA, http://webnet.oecd.org/hpv/ui/handler.axd?id=2080571c-ff2e-457a-b2d3-3a40d9ebb37c, 2017-03-19.

RAC (2018). Opinion proposing harmonised classification and labelling at EU level of butanone oxime. https://echa.europa.eu/documents/10162/fa6a43c5-6b2a-4c20-023b-801846dd19e3

Ryskova et al (1997). Genotoxicity of N-nitroso-N-methylurea and acetone oxime in the transgenic *Drosophila* carrying the human gene encoding a subunit of glutathione S-transferase. Folia Biologica (Praha), 43, 19-24.

Schulze, GE, Derelanko,M, (1993). Assessing the neurotoxic potential of methyl ethyl ketoxime in rats Fundamental and Appled Toxicology 21: 476-485.

Unpublished study report (1983). Dermal Sensitization Study: Guinea Pig Maximization Test, https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/5/2/?documentUUID=2be4d81a-311a-4ea8-bca1-2680d08e18df

Unpublished study report (1989a). Acute dermal toxicity study of Acetoxime in the rat. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/3/4/?documentUUID=c60efa7c-f4e8-48fc-8439-d43ac233af2d

Unpublished study report (1989b). Toxicological screening of Acetoxime. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/5/2/?documentUUID=1bc3f2cb-790e-4714-a255-cb6e40baa6b2

Unpublished study report (1990a). Primary dermal irritation study in rabbits. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/4/2

Unpublished study report (1990b). Eye Irritation study in rabbits. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/4/3

Unpublished study report (1990c). Guinea Pig Maximization Test. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/5/2

Unpublished study report (1990d). Acute In Vivo Cytogenetics Assay in Rats. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14908/7/7/3/?documentUUID=23f5d165-ae9c-4869-987b-0f7e476292f8.

Unpublished study report (1991a). Acute oral toxicity in rats. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/3/2/?documentUUID=22b79cbe-f3c7-4ddc-b0c2-e4f93fc651a7

Unpublished study report (1991b). Acute dermal toxicity study in rabbits. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/3/4

Unpublished study report (1991c). A subchronic (90-day) oral toxicity study with recovery in the rat with Acetone Oxime (158-888) via gavage. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/6/2/?documentUUID=964c8a00-9b59-4310-a574-3f817caba58e

Unpublished study report (2005a). “Wasox-MMAC2”: In vitro mammalian cytogenetic study (chromosome analysis). https://echa.europa.eu/de/registration-dossier/-/registered-dossier/17570/7/7/2/?documentUUID=71453b11-e19f-4a6c-b8cc-cd174d8665ad

Unpublished study report (2005b). “Wasox-VMAC2”: In vitro mammalian cytogenetic study (chromosome analysis). https://echa.europa.eu/de/registration-dossier/-/registered-dossier/26929/7/7/2/?documentUUID=2b5853d7-24a4-45f7-acaf-2500f684400a

Unpublished study report (2007). "Wasox-VMAC2": Micronucleus Test with Mice. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/26929/7/7/3

Unpublished study report (2012a). Acetonoxime Acute Dermal Toxicity Study in Rats. https://echa.europa.eu/registration-dossier/-/registered-dossier/5744/7/3/4/?documentUUID=9528072c-0bd4-490f-9e0c-9da5ffd9aff7

Unpublished study report (2012b). Acetonoxim: In vitro Mammalian Cell Gene Mutation Test: (Mouse Lymphoma Assay). https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/7/2/?documentUUID=515d2e94-e9d2-4db7-a27d-216eadc86824.

Unpublished study report (2013). Skin Senzitisation Test: Local Lymph Node Assay with Acetonoxime. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5744/7/5/2/?documentUUID=cac14b0d-7070-470c-b88a-12f982dec520

Unpublished study report (2016). Report on the Testing of Acetone oxime in the in vitro Alkaline Comet Assay. [Original study made available during SEV].

Thoolen B, ten Kate FW, van Diest PJ, Malarkey DE, Elmore SA, Maronpot RR (2012). Comparative Histomorphological Review of Rat and Human Hepatocellular Proliferative Lesions. J Toxicol Pathol. 2012 Sep; 25(3): 189–199.

Thoolen et a. (2010). Proliferative and Nonproliferative Lesions of the Rat and Mouse Hepatobiliary System, Toxicologic Pathology, 38: 5S-81S, 2010.

TL2 (1984). Acute inhalation toxicity study of MEKO. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14908/7/3/3

TL19 (1990), Developmental toxicity study in rabbits. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14908/7/9/3/?documentUUID=6105d4ce-428e-42ef-8be7-86aea4b1d0aa

Völkel et al (1999). Slow oxidation of acetoxime and methylethyl ketoxime to the corresponding nitronates and hydroxy nitronates by liver microsomes from rats, mice and humans. Toxicological Sciences, 47, 144-150.

15 ANNEX I: READ-ACROSS JUSTIFICATION

In the following section the read-across has been described according to the guidance for the analogue approach (ECHA, 2008) as well as ECHA (2017a).

In the present CLH report read-across using butanone oxime, Wasox-MMAC2 and Wasox-VMAC2 as source substances has been applied for the endpoints listed in the following table:

Table 28: Studies used for read-across

Endpoint	Source Substance	Study type and reference
Carcinogenicity	Butanone oxime	Key study Newton et al. (2001). A chronic inhalation toxicity/oncogenicity study of methyl ethyl ketoxime in rats and mice.
Mutagenicity	Butanone oxime	NTP (1999). Technical Report on the Toxicity Studies of Methyl Ethyl Ketoxime
	Butanone oxime	Key study CSR study (1990). Acute *In Vivo* Cytogenetics Assay in Rats.
	Reaction mass of propan-2-one-O,O'-(methoxymethylsilandiyl)dioxime; propan-2-one-O-(dimethoxymethylsilyl)oxime; propan-2-one-O,O',O''-(methylsilantriyl)trioxime Wasox-MMAC2, CAS 797751-43-0	Supportive study CSR study (2005a). Wasox-MMAC2: *In vitro* mammalian cytogenetic study (chromosome analysis)
	Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl]oxime Wasox-VMAC2, CAS 797751-33-0	Supportive study CSR study (2005b). Wasox-VMAC2: *In vitro* mammalian cytogenetic study (chromosome analysis)
	Wasox-VMAC2, CAS 797751-33-0	Supportive study CSR study (2007). Wasox-VMAC2: Micronucleus Test with Mice.
Narcotic effect	Butanone oxime	Key study Schulze and Derelanko (1993) cited in Germany (2017) Assessing the Neurotoxic Potential of Methyl Ethyl Ketoxime in Rats
		Key study TL2 (1984) cited in Germany (2017)

Endpoint	Source Substance	Study type and reference
		Acute inhalation toxicity study of MEKO.
		Key study Derelanko et al. (2003) cited in Germany (2017) Developmental toxicity studies of methyl ethyl ketoxime (MEKO) in rats and rabbits

15.1 Reliability and adequacy of the source studies used for read-across

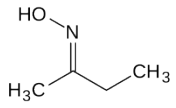
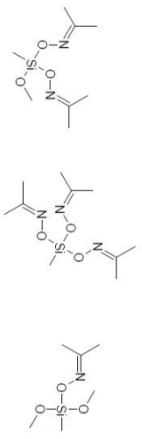
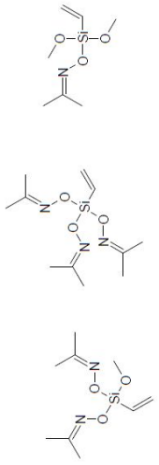
According to the ECHA (2008) Guidance “Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals, the used data needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy according to Chapter R.4 (“Evaluation of available information”).

For a detailed evaluation of the available data depicted in Table 28 please refer to the respective endpoint(s) in this document (Chapter 10.8, 10.9 and Chapter 10.11). The experimental studies for the analogue approach have been analysed for adequacy and reliability and are classified with Klimisch score 1 or 2.

15.2 Identity and characterisation of the source substances

The identity of the source substances is compiled in the following table:

Table 29: Chemical identity of the source substances

SUBSTANCE IDENTITY			
Public name:	Butanone oxime	Reaction mass of propan-2-one-O,O'-(methoxymethylsilandiyl)dioxime; propan-2-one-O-(dimethoxymethylsilyl)oxime; propan-2-one-O,O',O''-(methylsilantriyl)trioxime	Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl]oxime
IUPAC name	(2E)-N-Hydroxy-2-butanimin (Chemspider, 2017)[8] Butan-2-one oxime (Germany, 2014)	n.r.	n.r.
EC number:	202-496-6	460-110-3	458-680-3
CAS number:	96-29-7	797751-43-0	797751-44-1
Molecular formula:	C_4H_9NO	n.r. (multiconstituent substance)	n.r. (multiconstituent substance)
Molecular weight range [g/mol]:	87.122 g/mol	n.r. (multiconstituent substance)	n.r. (multiconstituent substance)
Synonyms:	MEKO, methylethyl ketoxime, 2-butanone oxime	WASOX-MMAC2	WASOX -VMAC2
Chemical structure:	 source: European Chemicals Agency http://echa.europa.eu/	 source: European Chemicals Agency, http://echa.europa.eu/	 source: European Chemicals Agency, http://echa.europa.eu/
Purity:	>99%*	n.r. (UVCB)	n.r. (UVCB)

* Germany (2014), n.r. (not reported)

[8] http://www.chemspider.com/Chemical-Structure.4481809.html

15.3 Link of structural similarities and differences with the proposed prediction (analogue approach):

In accordance with the ECHA Guidance (Chapter R.6), substances whose physico-chemical and/or toxicological and/or ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity, may be considered as a group or "category„ of substances. The similarities may be due to a number of factors (ECHA, 2008) e.g.

- Common functional group
- Common precursor or breakdown products
- Constant pattern in changing potency
- Common constituents or chemical classes

In the present read-across butanone oxime and acetone oxime have the same functional group (oxime group, imine group) and both are ketoximes. Butanone oxime has one additional methylene group compared to acetone oxime.

For mutagenicity Wasox-MMAC2 and Wasox-VMAC2, which are multicomponent substances containing one, two or three acetone oxime groups - with the difference that they are also methyl or vinyl substituted on the silicon atom - are used to support the data requirements. The substances undergo rapid hydrolysis (DT_{50} < 1 hour) in aqueous solution to acetone oxime and reactive methyl or vinyl substituted silanetriols. The methyl or vinyl silanetriols can condense to form substituted silanols, disilanols and siloxanes. However, it is unclear at which concentrations these chemicals are formed and no details on the composition or conditions was given by the registrant(s). Therefore, it is not clear if the condensation reactions produce only higher molecular weight siloxanes or if also other silanols are still present. OECD (2009) concluded that the mammalian toxicity profile of butanone oxime is similar to that seen for the methyl and vinly substituted oximino silanes containing three methylethylketoxime groups (that also hydrolyse rapidly in water to MEKO and reactive methyl or vinyl substituted silanetriols). However, though Wasox-MMAC2 and Wasox-VMAC2 release acetone oxime during hydrolyses also methyl- or vinyl-substituted silanetriols and condensed silanol material is formed, that may contribute to the overall toxicity.

Acute and repeated oral and dermal toxicity studies in rats with methyl/vinyl-MEKO-silane and methyl/vinyl-methyl isobutyl ketoxime-silane indicate that difunctional oxime silanes, containing both a methyl and a vinyl group caused degeneration of the seminiferous tubules of the testes. The testicular toxicity appears to be associated with the methyl/vinyl silane portion and not the oxime group of the oxime silane molecules (Derelanko and Rusch, 2008). Stable silanetriols have been found to reversibly inhibit the acetylcholinesterase activity at a 100 µM concentration *in vitro* (Blunder et al. 2011) indicating biological activity of this moiety. Therefore, as the methyl/vinyl silane moiety is not present in acetone oxime, these findings cannot be used to support read across for these hazard classes. However, the negative in vivo micronucleus test conducted with Wasox-VMAC2 can be used in the read across for the endpoint mutagenicity as it can be concluded that neither the oxime nor the silanol moieties produced micronuclei in this system.

A stepwise approach for applying read-across is set out in Chapter R.6 section 6.2.3 "Guidance on a stepwise procedure to perform the analogue approach" (ECHA, 2008). The outcome of the stepwise approach to perform the read-across from butanone oxime to acetone oxime for the endpoints mutagenicity, carcinogenicity and narcotic effects is provided in this Annex. For the endpoint mutagenicity the information requirement for structural and numerical chromosome aberrations for butanone oxime was used for read-across to acetone oxime supported by the read-across from Wasox-MMAC2 and Wasox-VMAC2 to acetone oxime.

The values obtained from the source substances were used in a way that the prediction constitutes a worst case (no underestimation of the effects that would be observed in a study with the target substance if it were to be conducted).

15.4 Bias that may influences the prediction

Butanone oxime has been investigated and evaluated for carcinogenicity in animal studies (Germany, 2014). While in principle two isomeric forms for butanone oxime (cis- and trans isomers) exists, the trans isomer predominates (>99%, according to Germany, 2014). The chemical structure of acetone oxime displays no isomeric forms. Though isomer specific effects of cis butanone oxime maybe possible, the very low amount <1% classifies butanone oxime as monoconstituent substance, like acetone oxime.

Because acetone oxime is the tautomeric form of 2–nitrosopropane, a reduction product of 2-nitropropane, another possible similar compound for the endpoint mutagenicity/carcinogenicity is 2-nitropropane, a genotoxic hepatocarcinogen in rats (NTP, 2000)[9]. In metabolism studies with acetone oxime (cf. section 9.1) propane 2-nitronate was experimentally determined *in vivo* in urine in rats as well as in *in vitro* liver microsome studies also with human hepatocytes (Kohl et al. 2002, Völkel et al 1999). However, the amounts were relatively small. Standard information requirements for mutagenicity for acetone oxime do not indicate a genotoxic potential. Please see also the following chapter “Hypothesis for the analogue approach”. Chemical structures of these substances and metabolites can be depicted from Figure 3.

Propane 2-Nitronate[10] Nitropropane[11] Nitrosopropane[12] Acetone oxime[13]

Figure 3: Chemical structures of similar nitro-compounds

[9] https://ntp.niehs.nih.gov/ntp/roc/content/profiles/nitropropane.pdf

[10] source: PubChem Identifier: CID 107791; https://pubchem.ncbi.nlm.nih.gov/compound/107791#section=2D-Structure

[11] source: PubChem Identifier: CID 398; https://pubchem.ncbi.nlm.nih.gov/compound/2-Nitropropane#section=Structures

[12] source: PubChem Identifier: CID 79121; https://pubchem.ncbi.nlm.nih.gov/compound/79121#section=2D-Structure

[13] source: PubChem Identifier: CID 67180; https://pubchem.ncbi.nlm.nih.gov/compound/Acetone-oxime#section=2D-Structure

15.5 Hypothesis for the analogue approach

Butanone oxime used as source substance

Endpoint: Carcinogenicity

Butanone oxime displays a high structural similarity to acetone oxime (see Figure 4).

Figure 4: Chemical structures of butanone oxime and acetone oxime

Both chemicals are ketoximes. The structural difference is that butanone oxime displays a methyl and an ethyl group. In the case of acetone oxime both alkyl groups are methyl groups. There is only one major isomer for butanone oxime (MEKO), which is trans/anti (>99%) according to Germany (2014). Acetone oxime has no isomers. The read-across approach is used for carcinogenicity (key study) in addition to other lines of evidence in a weight of evidence argumentation.

No species-specific mode of action for butanone oxime carcinogenesis was identified (RAC, 2018). Butanone oxime and acetone oxime can hydrolyse to butane and acetone, respectively as well as to possibly the common metabolite hydroxylamine. NTP (1999) confirmed as possible second hydrolyses product for butanone oxime hydroxylamine.

Also both substances can be converted to a minor degree to butane 2-nitronate and propane 2-nitronate (P2-N), respectively. The involvement of reactive metabolites/oxygen and/or nitrosating species in the aetiology of the observed effects that may lead to carcinogenicity, however, remains unclear.

The QSAR prediction from the QSAR Toolbox V3.3.5 gave the same endpoint specific structural alert for the source and the target compound: Category: Oncologic primary classification C-Nitroso and Oxime Type (cf. section 10.9).

RAC (2018) stated that there is limited evidence to propose a mode of action that involved cytotoxicity for the increased incidences of liver tumours observed in rats and mice.

Endpoint: Mutagenicity

Please refer for structural similarities and metabolism of the source chemical butanone oxime and the target acetone oxime to the section above. Butanone oxime and acetone oxime did not induce gene mutations in bacterial reverse mutation assays (cf. Chapter 10.8). Based on *in vitro* and *in vivo* mutagenicity data, Germany (2014) concluded that there was no evidence of germ cell mutagenicity of butanone oxime in standard mutagenicity or genotoxicity tests. Also results from standard mutagenicity or genotoxicity tests on acetone oxime were negative. However both substances produced RNA adducts, for acetone oxime also DNA modifications in rats (*in vivo*) were shown (cf. Chapter 10.8).

The QSAR prediction from the QSAR Toolbox V3.3.5[14] gave no general mechanistic structural alert for DNA binding for the source and the target compound. DNA binding is one mechanism well linked to carcinogenicity and genotoxicity. However an endpoint specific structural alert for *in vivo* mutagenicity (micronucleus): “H acceptor-path3-H acceptor” for both substances was identified indicating that possibly the chemical can interact with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding.

[14] http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm

For the source as well as for the target substance the involvement of sulfotransferase mediated formation of DNA reactive nitrenium ions have been discussed as well as the formation of respective nitronates (probable intermediate for the generation of reactive oxygen species) as a possible mechanism for the induction of liver tumours (Germany 2014, Kreis et al. 2000, Kohl et al. 2002, Völkel et al. 1999). The toxicokinetic results from butanone oxime indicate that butane 2-nitronate formation alone is not sufficient to explain the carcinogenicity of butanone oxime (Germany, 2014). *In vitro* experiments with mice and rats liver microsomes and human hepatocytes indicate that acetone oxime is metabolized to the corresponding nitronate at rates 50% of those observed with butane oxime oxidation (Völkel et al. 1999). Results from *in vitro* and *in vivo* metabolism studies with acetone oxime suggest that amounts of P2-N (or 2-NP) were relatively small (Kohl et al. 1992). Though biotransformation of acetone oxime *in vivo* in rats to propane 2-nitronate (anionic form of 2-NP) and *in vitro* to 2-NP was shown, it is also generated as a product of the metabolic detoxification (reduction) of the nitronate (Kohl et al. 2002, Völkel et al. 1999 and Haas-Jobelius et al. 1991).

While 2-NP and P2-N were substrates for rat and human sulfotransferases in *in vitro* cell cultures this was not the case for acetone oxime (Andrae et al. 1999, Kreis et al. 1998, Kreis et al. 2000).

In addition to butanone oxime also Wasox-MMAC2 and Wasox-VMAC2 were considered as source substances for the read-across. Both substances releases one, two or three moles of acetone oxime and one mole of reactive methyl or vinyl substituted silanetriol. Due to rapid abiotic transformation, only low systemic exposure to parent compounds Wasox-MMAC2 and Wasox-VMAC2 is likely to occur. From the hydrolysis study[15] (preliminary test according to EU Method C.7 and GLP) with Wasox-MMAC2 and Wasox-VMAC2 the half-lives of the 3 main components of the test substances at 25°C and at pH 4, pH 7 and pH 9 were shorter than 1 hour in each case. Therefore, the experimental data on these source substances can be used to predict effects also caused by acetone oxime. The condensed high molecular weight silanetriols are not considered to be very biologically active, however it is unclear if methyl and/or vinyl substituted silanetriol contribute to toxicological effects. Experimental *in vivo* data in rats showed that testicular toxicity appears to be associated with the methyl/vinyl silane portion and not the oxime group (Derelanko and Rusch, 2008). Stable silanetriols have been found to reversible inhibit the acetylcholinesterase activity at a 100 µM concentration *in vitro* (Blunder et al. 2011) indicating biologically activity of the moiety. Therefore, this read-across is only used to support the no classification of acetone oxime for mutagenicity.

Endpoint: Transient Narcosis

Please refer for structural similarities and metabolism of the source chemical butanone oxime and the target acetone oxime to the section above.

According to literature transient narcosis is a common effect in laboratory animals for low molecular weight oxime compounds (Derelanko and Rusch, 2008). For butanone oxime narcotic effects are described in single/acute and repeated dose/sub-chronic exposure situations; for acetone oxime effects are reported only in acute toxicity studies:

After oral administration in SD rats at 1000 mg/kg and 3000 mg/kg ataxia after dosing and hypoactivity were reported (unpublished study report, 1991a). Hypoactivity was also observed in the high dose group at 1000 mg/kg in rabbits after acute dermal exposure (unpublished study report, 1991b), but this could also be possibly attributed to the compromised health status of the animals. In a supportive study in rats in all dosed animals (100, 300 and 1000 mg/kg) lethargy was reported (unpublished study report, 1989a).

The data base for butanone oxime is more extensive and the reported dose levels for transient narcotic effects on an acute basis are lower compared to acetone oxime. Oral single doses of ≥ 300 mg/kg bw butanone oxime were found to produce transient and reversible changes in neurobehavioral function consistent with CNS depression, but no evidence of cumulative neurotoxicity was detected (neurotoxicity study, Schulze and Derelanko, 1993; Germany, 2014). After subchronic exposure transient treatment-related changes in ease of cage removal, ease of handling, and in posture, gait, and aerial righting were observed at the 400 mg/kg/d (Schulze and Derelanko, 1993). The highest dose

[15] https://echa.europa.eu/registration-dossier/-/registered-dossier/17570/5/2/3

tested in the repeated dose toxicity study with acetone oxime was 250 mg/kg/d, no clinical signs indicative of narcosis were reported (unpublished study report, 1991c).

In the developmental study with butanone oxime in rabbits (dams) decreased activity and wobbly gait occurred at much lower dose levels at ≥ 40 mg/kg bw/d (Derelanko et al. 2003, Germany et al. 2017). In the acute inhalation toxicity study a strong but transient narcotic effect occurred in both sexes at 4.83 mg/L/4h during the exposure (TL2, 1984, Germany et al. 2017). No developmental or acute inhalation toxicity study with acetone oxime is available.

The read across is used to support the available data with acetone oxime. However, the role of metabolism/hydrolysis and a contribution of the metabolite acetone regarding these effects might also be possible.

No mode of action is described for the narcotic effects of butanone oxime in Germany (2014) or RAC (2018). Also no mechanistic information on acetone oxime is available that details the key events for this endpoint. However narcosis was also observed after inhalation administration of another oxime, acetaldehyde oxime to Wistar rats (OECD, 2006).

The structural similarities between the source and the target including the common functional oxime group justifies to consider the narcotic effects observed for butanone oxime also for acetone oxime in addition to the experimental evidence from the target chemical.

Purity/impurities

The purity of the analogue substance butanone oxime is according to Table 29 information very high (above 99%). Impurities are thus not likely to influence the overall toxicity. Wasox-MMAC2 and Wasox-VMAC2 are multicomponent mixtures; no information on impurities was available.

Physico-chemical properties similarity

Acetone oxime and butanone oxime are low molecular weight compounds with a shared oxime group. They all have a high water solubility, low partition coefficient octanol/water (Kow) and are stable at higher pH values and have moderate to high vapour pressures (cf. Table 30). The vapour pressure of acetone oxime (estimated: 242 Pa at 25°C) is comparable to butanone oxime (two values available: 1070 Pa at 20°C and 140 Pa at 20°C). While for the first value the exact method is not known ("equivalent or similar to OECD Guideline 104") the second value is cited in NTP and according to Germany (2014) also in studies of US EPA and of Environment Canada. Therefore, it could be assumed that this value should also be valid according to Germany (2014).

Given all of the above evidence, it is considered appropriate to conclude that, whilst there are differences in some physico-chemical parameters, acetone oxime is qualitatively similar to butanone oxime with respect to most parameters.

Wasox-MMAC2 and Wasox-VMAC2 are multicomponent mixtures that hydrolyse fast (DT_{50} <1 hours) to acetone oxime and reactive methyl or vinyl substitutes silanetriol. Therefore the physical chemical properties, Kow and vapour pressure could not be measured according to the information provided by the registrants, data waiving was used for water solubility.

Based on these physico-chemical properties compiled in Table 30 and resulting behaviour of the analogue, it is justified that butanone oxime is an appropriate reference material for read-across.

Mammalian toxicological data

As depicted in Table 31 butanone oxime, acetone oxime, Wasox-MMAC2 and Wasox-VMAC2 have some similar toxicological patterns with regard to mammalian toxicological endpoints.

Concerning local effects acetone oxime and butanone oxime were severe eye irritants and slight irritating effects on skin were observed in animal studies. For acute dermal toxicity it seemed that rabbit was more sensitive compared to rats for acetone oxime and butanone oxime. The acute dermal LD_{50} value in rats was > 2000 mg/kg bw (unpublished study report, 2012a) compared to an LD_{50} in rabbits that was < 2000 mg/kg bw (range > 1000 < 2000 mg/kg bw) (unpublished study report, 1991b)

indicating that rabbit is more susceptible to effects of acetone oxime. While more data are available for butanone oxime from single dose studies in rats and from repeated dose studies in rabbits, rabbits appear more sensitive than rats to the acute toxic effects of butanone oxime (Germany, 2014). The data from the other analogues do not allow drawing a conclusion on species differences.

For acetone oxime and butanone oxime transient narcotic effects are described after acute oral, dermal or inhalation exposure studies.

In repeated or chronic dose studies the determined effect values are in the same range for all the analogue substances if exposure duration is taken into consideration. The target is the hematopoietic system which is consistent with haemolytic anemia, methemoglobin formation and compensatory responses such as reticulocytosis, extramedullary hematopoiesis, splenic and hepatic hemosiderin pigment accumulation and increased spleen and liver weights.

Mutagenicity studies showed mixed results for Wasox-VMAC2 in *in vitro* systems. *In vivo* indicator studies concerning DNA and RNA adduct formation in rat liver were available with acetone oxime and butanone oxime. For butanone oxime no DNA modifications were detected. In liver RNA from butanone oxime exposed rats, a dose, sex and time-dependent formation of 8-aminoguanosine and 8-oxoguanosine was observed (Germany, 2014).

For acetone oxime the main identified DNA and RNA lesion in male and female rats were 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanine. Also a higher DNA adduct formation in males compared to females occurred (Hussain et al. 1190; Guo et al. 1990). A mammalian erythrocyte micronucleus test *in vivo* with Wasox-VMAC2 did not produce relevant increases of the numbers of micronuclei in polychromatic erythrocytes. In a further second *in vivo* chromosome aberration assay with butanone oxime in rats no significantly increased chromosomal aberrations in the bone marrow occurred. Based on standard information mutagenicity and genotoxicity test acetone oxime and butanone oxime are not expected to induce directly heritable mutations in mammals (cf. Table 31).

Table 30: Data matrix for the analogue read-across: physico-chemical properties

Substances	**Acetone oxime**	**Butanone oxime[1]**	**Wasox-MMAC2[2, **]**	**Wasox-VMAC2[2, ***]**
Read-across	**Target chemical**	**Source chemical**	**Source chemical**	**Source chemical**
State of substance at 20°C and 101.3 kPa	White solid	Liquid	Yellowish, clear liquid	Yellowish, brownish liquid
Melting point	60°C (measured)	-29.5°C (measured data)	35 ºC (measured data)	-5 ºC (acetoneO,O'-[methoxy(vinyl)silanediyl]oxime); 12.9 ºC (acetoneO,O',O''-(vinylsilanetriyl)oxime) -25.6 ºC (acetoneO-[dimethoxy(vinyl)silyl]oxime), (estimated values)
Boiling point	134°C*	>152°C (at 1013kPa)	Decomposition before boiling at about 190°C. The decomposed test substance boiled from about 205°C on.	Decomposed before and during boiling (205 ºC). Decomposed substance boiled from 220 ºC
Relative density	1.06 at 20°C*	0.92 at 20˚C*	1.01 at 20ºC	1.02 at 20ºC
Vapour pressure	242 Pa at 25°C (measured) 164 Pa at 25°C (estimated)	1070 Pa at 20°C 140 Pa at 20°C	not possible to determine the vapour pressure	not possible to determine the vapour pressure
Dissociation constant pKa:	12.42 at 24.9°C	12.45 at 25°C (measured data)	n.r.	n.r.
Water solubility	Very soluble in water	100000 mg/L at 25°C and pH 7 (measured data)	Not applicable since the substance is hydrolytically unstable (half-life <12h)	Not applicable since the substance is hydrolytically unstable (half-life <12h)
Partition coefficient octanol/	0.077	0.63 at 25°C (estimated)	Experimental determination not possible due to hydrolytically	Experimental determination not possible due to hydrolytically

water			unstable (half-life <12 h)	unstable (half-life <12 h)
Hydrolysis	Half-life of 18 days at neutral pH (QSAR estimation). Acetone and hydroxylamine (potentially as salt) are expected as hydrolysis products.	Experimental result: 14% hydrolysis at 20 °C was obtained after 4 days at pH 7. The hydrolysis products are methyl ethyl ketone and a hydroxylamine salt*	Experimental result: DT50 <1 hour at 2 °C (EU Method C.7, GLP study)**	Experimental result: DT50 <1 hour at 25°C (EU Method C.7, GLP study)***
Biodegradation	Experimental results: Not readily biodegradable (OECD 301D, GLP study)	Experimental results: Not readily biodegradable; Inherently biodegradable*	Experimental results: Not readily biodegradable (OECD 301B, GLP study)	Experimental results: Not readily biodegradable (OECD 301B, GLP study)

n.r. (not reported)
Information source: [1]Germany 2014, [2]Chemcial safety report (CSR) on acetone oxime *https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/1
** https://echa.europa.eu/registration-dossier/-/registered-dossier/17570/5/2/3, *** https://echa.europa.eu/de/registration-dossier/-/registered-dossier/26929/1

Table 31: Data matrix for the analogue read across: mammalian toxicity

Substances	**Acetone oxime**	**Butanone oxime[2]**	**Wasox-MMAC2[3, **]**	**Wasox-VMAC2[3, ***]**
Read-across	**Target chemical**	**Source chemical**	**Source chemical, supportive**	**Source chemical, supportive**
Acute Toxicity: Oral (for more details see Chapter 10.11)	LD_{50} >3000 mg/kg (rats, GLP) Ataxia	LD_{50} = 2326 mg/kg (male rats, OECD 401) LD_{50} >900 mg/kg (m/f rats, acute neurotoxicity study) LD_{50} = 160 mg/kg <320 mg/kg (rabbits, OECD 414)	Experimental results: LD_{50} >2000 mg/L (rat, female) (OECD 423, GLP study)	Experimental results: LD_{50} >2000 mg/L (rat, female) (OECD 423, GLP study)
Acute Toxicity: Inhalation (for more details see Chapter 10.11)	No data	LC_{50} assumed to be higher than 13.2 mg/L/4h (likely >20 mg/L/4h, vapour, rats, in-house protocol) Signs of narcosis LC_{50} (4h) >4.83 mg/L (GLP study)	No data	No data
Acute Toxicity: Dermal	LD_{50} >2000 mg/kg (rats, OECD 402, GLP) LD_{50} >1000 mg/kg, (rabbit, ~OECD 402, GLP study, hypoactivity)	LD_{50} >1000 mg/kg (rabbit, OECD 402) LD_{100} = 1848 mg/kg bw (rabbit, EPA OTS 798.1100)	LD_{50} >2000mg/L (rat, male/female) (OECD 402, GLP study).	LD_{50} >2000mg/L (rat, male/female) (OECD 402, GLP study).
Skin irritation	Slight skin irritant (~OECD 404, GLP study)	Slight skin irritant (no specified test method) No irritating (OECD 404)	Non-irritant (OECD 404, GLP study)	Non-irritant (OECD 404, GLP study)
Eye irritation	Irreversible effects on the eye (GLP study, ~OECD 405)	Irreversible effects on the eye (OECD 405)	Non-irritant (rabbits) (OECD 405, GLP study)	Non-irritant (rabbits) (OECD 405, GLP study)

Substances	**Acetone oxime**	**Butanone oxime[2]**	**Wasox-MMAC2[3, **]**	**Wasox-VMAC2[3, ***]**
Skin Sensitization	Positive GPMT study(OECD 406, GLP study) Negative in LLNA in mice (OECD 429, GLP study)	Positive GPMT study (OECD 406, GLP) Negative in LLNA in mice (OECD 429)	No skin sensitizer (Local Lymph Node Assay, OECD 429, GLP study).	No skin sensitizer (Local Lymph Node Assay, OECD 429, GLP study).
Repeated Dose Toxicity	NOAEL = 10 mg/kg bw/d (rat, OECD 408) target: hematopoietic system; anemia, elevated methemoglobin level, regenerative anaemia, compensatory reticulocytosis, erythrocytic morphology consistent with polychromia and occasional Howell-Jolly bodies; effects in the spleen and liver (extramedullary hematopoiesis, increased organ weights). Elevated liver weights in high dose males and spleen weights in mid- and high dose group. Hepatocellular changes were more severe in treated male rats than in female rats.	LOAEL = 10 mg/kg bw/d (rat, rabbit, mouse) target: hematopoietic system in rats, rabbits, and mice; neurobehavioral effects in rats and rabbits; degeneration of the nasal olfactory epithelium in rats and mice; hyperplasia of the urinary bladder transitional epithelium in mice. The lowest oral LOAEL of 10 mg/kg bw/d, based on effects in the spleen and liver of adult rats observed in a two-generation reproduction study. In adult female rabbits signs of anemia at this dose in a range-finding developmental study (Germany, 2014)	28 day-NOEL = 20 mg/kg bw/day (rats, male/female) based on effects on the hematopoietic system, extramedullary, liver and spleen weight changes, haematopoiesis in the spleen and hypercellularity in the bone marrow (OECD 408, GLP study)	28 day-NOEL = 20 mg/kg bw/day (rats, male/female) (haemolytic anemia). Test substance related alteration in liver and spleen weight; Histopathologically, extramedullary haematopoiesis in the spleens of all high dosed animals (200mg/kg/d) and most mid dosed animals at 63 mg/kg/d. Hypercellularity of the bone marrow occurred. (OECD 407, GLP study)
Gene mutation in bacteria (in vitro)	Negative (+/-S9) in *Salmonella typhimurium* TA97, TA98, TA100, TA1535. (OECD 471, GLP study)	Negative (+/-S9) in several standard bacterial strains (OECD 471)	Negative (+/-S9) in *Salmonella typhimuriumTA97a, TA98, TA100, TA102 and TA1535.* (OECD 471, GLP study)	Negative (+/-S9) in *Salmonella typhimuriumTA97a, TA98, TA100, TA102 and TA1535* (OECD 471, GLP study)
Chromosomal aberration (in-	Read-across (no data)	In cytogenetic tests no induction of SCE was observed up to	Negative (+/-S9) in human lymphocytes.	Positive without metabolic activation with 20h treatment;

Substances	**Acetone oxime**	**Butanone oxime[2]**	**Wasox-MMAC2[3, **]**	**Wasox-VMAC2[3, ***]**
vitro)		cytotoxicity (500 µg/mL) in the absence of S9 or up to the assay limit (5000 µg/mL) in the presence of S9. No increase in chromosomal aberrations was observed in cultured CHO cells treated with up to 5000 µg/mL butanone oxime +/- S9.	(OECD 473, GLP study)	negative (+/-S9; 3h treatment) in human lymphocytes. (OECD 473, GLP study)
Mammalian gene mutation (in vitro)	Negative mammalian cell gene mutation assay in mouse lymphoma cells (OECD 476, GLP)	Mouse lymphoma study (OECD 476) found evidence of mutagenic activity in mouse lymphoma L5178Y cells in the absence of S9 activation but in the presence of cytotoxicity (growth inhibition of 50-92.5% at doses of 2.8-6.5 µL/mL).	No data	No data
Indicator mutagenicity tests	-Negative in unscheduled DNA Synthesis (UDS) assays in V79 cell lines, OSV cells and rat primary hepatocytes -Negative in-vitro alkaline comet assay	-Negative in unscheduled DNA Synthesis (UDS) test in rat primary hepatocytes -No SCE induction (see above)	No data	No data
Genetic Toxicity in vivo	-Detection of DNA and RNA adducts in rat liver -Positive in the SMART assay (*Drosophila melanogaster*)	-*Drosophila melanogaster* sex-linked recessive lethal test (~OECD 477): negative -Chromosome aberration test (~OECD 475): negative -Mammalian Erythrocyte Micronucleus test (~OECD 474): negative	No data	Negative (Mammalian Erythrocyte Micronucleus Test, OECD 474, GLP study).

Substances	**Acetone oxime**	**Butanone oxime[2]**	**Wasox-MMAC2[3, **]**	**Wasox-VMAC2[3, ***]**
		-RNA and DNA adducts in liver (rat): only RNA adducts detected		
Carcinogenicity	Read-across Supporting: LOAEL ≤ 1000 ppm Incidence of liver tumours (hepatocellular adenomas) in male rats was 80% at week 93 (statistically different to 0% in the control); (no guideline, Klimisch 3)	**Positive** 75 ppm (270 mg/m^3) for tumour development, Tumours in the liver (adenomas and carcinomas) in rats and mice. Statistically significant increases in incidence at 75 ppm for liver adenomas in male rats and at the highest concentration of 374 ppm for liver carcinomas in male rats and mice. Increased incidence of liver adenomas occurred also in female rats and mice in the high concentration group, but no statistically significance. Statistically significant increase of mammary gland fibroadenomas in male rats at the 374 ppm. A NOAEC for carcinogenicity was not derived for rats and mice (Germany, 2014).	No data	No data

Information source: [1]OECD 2006, [2]Germany 2014, [3]Chemcial safety reports (CSR) on acetone oxime *https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/1 ** https://echa.europa.eu/registration-dossier/-/registered-dossier/17570/7/1, ***https://echa.europa.eu/de/registration-dossier/-/registered-dossier/26929/7/1