Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Acetone oxime

EC Number: 204-820-1
CAS Number: 127-06-0

CLH-O-0000007091-82-01/F

Adopted

18 March 2022

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

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CLH-O-0000007091-82-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Acetone oxime**

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

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

The proposal was submitted by **Austria** and received by RAC on **8 April 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **5 July 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 September 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

Co-Rapporteur, appointed by RAC: **Ruth Moeller**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2022** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 submitters proposal	TBD	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	
RAC opinion	TBD	Acetone oxime	204-820-1	127-06-0	Carc. 1B Acute Tox. 4 STOT SE 3 STOT RE 2 Eye Dam. 1 Skin Sens. 1	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	
Resulting Annex VI entry if agreed by COM	TBD	Acetone oxime	204-820-1	127-06-0	Carc. 1B Acute Tox. 4 STOT SE 3 STOT RE 2 Eye Dam. 1 Skin Sens. 1	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	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Acetone oxime (Figure 1) is used as anti-skinning agent for the preparation of coatings/printing inks. It is also used as an intermediate for the manufacture of other substances/products, mainly in the manufacture of oxime silanes which are applied as cross-linkers for silicon sealants. Consumer uses were not registered, but exposure of the general population can be expected via the use of paints, printing inks and silicon sealants in non-industrial settings.

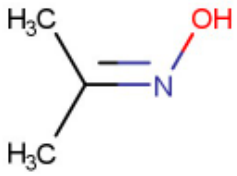


Figure 1: Structural formula of acetone oxime

Besides the proposal for harmonised classification of acetone oxime for carcinogenicity, harmonised classification is also proposed for other hazard classes due to differences in self-classification and due to the dossier submitter (DS) disagreeing with the current self-classification by the active registrants.

The substance has no harmonised classification, but it has different self-classifications 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) according to the C&L inventory [accessed in December 2020].

Toxicokinetics

No toxicokinetic study according to an OECD test guideline (TG) is available for acetone oxime. Thus, physico-chemical properties, QSAR estimates and information from the analogue butanone oxime were considered.

In vitro and *in vivo* metabolism studies indicate that acetone oxime is converted in liver tissue of rats, mice and humans to propane 2-nitronate (P2-N), most likely by activation of cytochrome P450 enzymes. 2-nitropropane (2-NP), a genotoxic hepatocarcinogen, is formed in tautomeric equilibrium with P2-N. Amounts of P2-N and its neutral tautomer 2-NP were reported to be small in *in vitro.* In *in vivo* studies P2-N and acetone oxime were excreted in comparable, although small, amounts in urine. *In vitro* experiments with mice and rat liver microsomes and human hepatocytes indicated that acetone oxime is metabolized to the corresponding nitronate at rates of 50% of those observed with butane oxime oxidation (to yield the butanone nitronate) (Völkel *et al.*, 1999). However, following detoxification, 2-NP can also undergo cellular reduction to acetone oxime.

Acetone oxime was shown not to be a substrate for rat and human sulfotransferases but these enzymes were demonstrated 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 QSAR predictions for the hydrolysis of acetone oxime, it was postulated that an additional metabolic

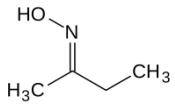
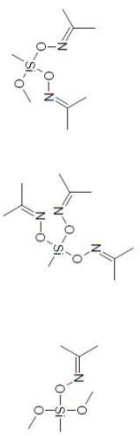
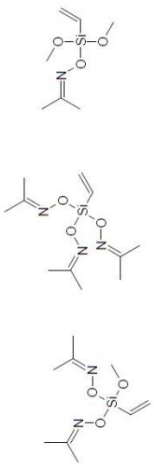
pathway could be the hydrolysis of acetone oxime to acetone, hydroxylamine and CO_2. The hydrolysis may occur both enzymatically and non-enzymatically.

Read-across proposed by the DS

The DS proposed read-across for the hazard classes STOT SE (H336, narcotic effects), mutagenicity and carcinogenicity using data from the following source substances: the structurally similar oxime, butanone oxime, and the two acetone oxime-releasing silanes Wasox-MMAC2 and Wasox-VMAC2.

Substance ID and structural similarity

***Table 1**: 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#) 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	797751-33-0	797751-44-1
Molecular formula:	C_4H_9NO	multiconstituent substance	multiconstituent substance
Molecular weight range [g/mol]:	87.122 g/mol	multiconstituent substance	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), # from http://www.chemspider.com/Chemical-Structure.4481809.html

Butanone oxime and acetone oxime have the same functional group (oxime group, imine group) and both substances are ketoximes (Table 1). Butanone oxime has one additional methylene group than acetone oxime. In the case of acetone oxime, both alkyl groups are methyl groups. There is only one major isomer for butanone oxime, which is trans/anti (>99%) according to Germany (2014), while acetone oxime has no isomers. The purity of the analogue substance butanone oxime is very high (above 99%) according to the information provided in Table 1. Impurities are thus not likely to influence the overall toxicity of butanone oxime.

Wasox-MMAC2 and Wasox-VMAC2 are multicomponent substances containing one, two or three acetone oxime groups, depending on the mixture composition, and are methyl or vinyl substituted on the silicon atom (Table 1). The substances undergo rapid hydrolysis (DT50 < 1 hour based on first measurement conducted after 45 minutes) in aqueous solution and hydrolysis products are acetone oxime and the respective highly reactive methyl or vinyl substituted silanetriol. The latter can condense and form large substituted silanols, disilanols and siloxanes. However, it was not reported at which concentrations the condensations occur and whether the reactions are complete. Therefore, it is not clear, if the condensation reactions yield only higher molecular weight siloxanes or if also other silanols (monomers, oligomers) are present. Wasox-MMAC2 and Wasox-VMAC2 are multicomponent mixtures, which is why no information on impurities is available.

Physico-chemical properties

Acetone oxime and butanone oxime are low molecular weight compounds with a high water solubility and a low partition coefficient octanol/water (Kow). Both substances are stable at higher pH values and have moderate to high vapour pressures (see Table 30 in Annex I of the dossier). The vapour pressure of acetone oxime is estimated to be 242 Pa at 25°C, while there are two values available for butanone oxime (1070 Pa at 20°C and 140 Pa at 20°C). The first value was derived without reporting the exact method ("equivalent or similar to OECD Guideline 104") and the second value is cited in NTP. According to Germany (2014), the second value is also cited in studies of the US EPA and Environment Canada. Therefore, it may be assumed that the second value derived by the NTP is valid. The DS concluded that although there are differences in some physico-chemical parameters acetone oxime is qualitatively similar to butanone oxime with respect to most of the physico-chemical parameters (Tab. 30 in Annex I of the dossier).

Wasox-MMAC2 and Wasox-VMAC2 are multicomponent mixtures that hydrolyse to acetone oxime and the reactive methyl or vinyl substituted silanetriols. Therefore, the physical chemical properties, K_{ow} and vapour pressure could not be measured according to the information provided by the registrant(s), and data waiving was used for water solubility (Tab. 30 in Annex I of the CLH dossier). The substances, however, undergo rapid hydrolysis upon contact with water/moisture/humidity and release acetone oxime, as well as the reactive methyl or vinyl substituted silanetriols.

Due to the quick release of acetone oxime upon hydrolysis, the DS considered the read-across from Wasox-MMAC2 and Wasox-VMAC2 to acetone oxime as plausible, although the physico-chemical properties of the silanes per se are not necessarily qualitatively similar to those of acetone oxime.

Comparison of mammalian toxicological data

As depicted in Table 31 in Annex I of the CLH dossier, butanone oxime and acetone oxime have similar toxicological patterns with regard to mammalian toxicological endpoints.

Concerning local effects, acetone oxime and butanone oxime were identified as severe eye irritants and both substances elicited slight irritating effects on the skin of animals.

With respect to the acute toxicity of acetone oxime and butanone oxime, rabbits seem to be the more sensitive/susceptible species when compared to rats. While species differences seem more pronounced in studies with butanone oxime (oral LD_{50}: 930 mg/kg bw in rats; converted acute toxicity point estimate (cATpE; OECD TG 414) in (pregnant) rabbits: 100 mg/kg bw), slight differences were observed with regards to the acute dermal toxicity of acetone oxime (dermal LD_{50} in rats > 2000 mg/kg bw, in rabbits between > 1000 mg/kg bw and < 2000 mg/kg bw). The available data for the oral acute toxicity of acetone oxime and the dermal acute toxicity of butanone oxime, as well as information on the other two analogues do not allow drawing conclusions on species differences, as only data on rats is available (oral and dermal LD_{50} > 2000 mg/kg bw).

For acetone oxime and butanone oxime, transient effects are described after acute oral, dermal or inhalation exposure that may resemble narcosis. Reported dose levels for transient narcotic effects on an acute basis are lower with butanone oxime compared to acetone oxime.

From repeated dose or chronic toxicity studies, the determined effect values with respect to the observed haemolytic anaemia, methaemoglobin (MetHb) formation and the secondary effects in spleen and liver are in the same range for all of the analogue substances, if exposure duration is taken into consideration (N/LOAELs for acetone oxime and butanone oxime: 10 mg/kg bw/d in 90-day repeated dose toxicity studies, NOAELs for Wasox-MMAC2 and Wasox-VMAC2: 20 mg/kg bw/d in 28-day repeated dose toxicity studies).

Butanone oxime and acetone oxime did not induce gene mutations in bacterial reverse mutation assays and 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 available results from standard mutagenicity or genotoxicity tests on acetone oxime were negative. Both substances were suggested to produce RNA adducts. For acetone oxime DNA modifications in rats (*in vivo*) were also reported. According to the DS, for both substances no structural alert for DNA binding but an endpoint specific structural alert for *in vivo* mutagenicity (micronucleus) was predicted from the QSAR Toolbox V3.3.514, "H acceptor-path3-H acceptor" was identified indicating that the chemical can possibly interact with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding. Wasox-MMAC2 and Wasox-VMAC2 were also considered as source substances for the read-across as both substances release one, two or three moles of acetone oxime and one mole of reactive methyl or vinyl substituted silanetriol after rapid abiotic transformation. Experimentally, testicular toxicity has been attributed to methyl/vinyl silane portion and inhibition of acetylcholinesterase activity was found for the stable silanetriols. Therefore, the data was only used to support no classification of acetone oxime for germ cell mutagenicity.

<u>Uncertainties regarding the read-across</u>

While there are in principle two known isomeric forms for butanone oxime (cis- and trans isomers), the trans isomer was reported to predominate (>99%, according to Germany, 2014). The chemical structure of acetone oxime displays no isomeric forms. Though isomer specific effects of cis-butanone oxime may be possible, its very low amount (< 1 %) classifies butanone oxime as mono-constituent substance, like acetone oxime.

In addition, the role of metabolism/hydrolysis of acetone oxime is not entirely clear and a contribution of the metabolite acetone to the local and systemic toxicity of acetone oxime might be possible.
Because acetone oxime is the tautomeric form of 2–nitrosopropane, a reduction product of 2-nitropropane (2-NP) (see also Toxicokinetics section), another possible similar compound for the endpoint mutagenicity/carcinogenicity is 2-NP, a genotoxic hepatocarcinogen in rats (NTP, 2000).

In metabolism studies with acetone oxime, relatively small amounts of propane 2-nitronate (P2N; tautomeric form of 2-NP) were experimentally determined *in vivo* in urine of exposed rats, as well as *in vitro* in liver microsome studies with human hepatocytes (Kohl *et al.* 2002, Völkel *et al.*1999).

Studies used for read-across

The following studies were used for read-across and are described in more detail in the respective hazard class paragraphs. All listed studies used for the analogue approach have been analysed by the DS for adequacy and reliability and were assigned a Klimisch score of 1 or 2.

Table 2: *Studies used for read-across by the DS*

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	Supportive study 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 797751-44-1	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) 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

RAC assessment of the proposed read-across

RAC agrees with the DS that acetone oxime and butanone oxime are structurally very similar and, in addition, both have rather similar relevant physico-chemical properties.

The data available for the source substance, butanone oxime, is considered relevant and reliable for the purpose of read-across and both the target as well as the source substance possess (only) one identical functional group, the oxime/imine group. The purity profile of both registered substances is reported to be high, but uncertainties arise from lacking information on the purity of acetone oxime used as test substance in numerous of the available *in vitro* and *in vivo* studies. Information on impurities is not available.
Additional uncertainties were identified with regards to the different metabolites of acetone oxime and butanone oxime, respectively (see also section on toxicokinetics below). For the latter substance, CO_2 and methyl ethyl ketone (MEK) were identified as main metabolites after hydrolysis, and butanone oxime was found to be oxidised to butane-2 nitronate by microsomal monooxygenases at very low rates. No sex differences in the rates of microsomal oxidation were noted. A third minor pathway was discussed for butanone oxime, i.e. the reduction of the substance. According to NTP (1999), there is also some limited evidence that the ketoxime is metabolized to the ketone and, presumably, hydroxylamine.
For acetone oxime, on the other hand, propane 2-nitronate (P2-N) and its neutral tautomer the genotoxic hepatocarcinogen 2-nitropropane (2-NP) were reported to be formed in small amounts in liver tissue *in vitro* and *in vivo (*50% of respective nitronate observed with butane oxime oxidation; Völkel *et al.*, 1999). However, 2-NP can in turn undergo cellular reduction to acetone oxime. Oxidation was suggested as another (potentially main) metabolic pathway. However, data is not available for acetone oxime, and the assumption was based only on metabolism studies with the analogue substance butanone oxime and the hydrolysis QSAR prediction for acetone oxime itself. Overall, it remains unclear, whether differences in toxicity profiles can be expected due to the different metabolite profiles of the two substances.

Regarding mammalian toxicity, RAC is of the opinion that hazard patterns are comparable with respect to acute and local toxicity including irritation and skin sensitisation of acetone oxime and butanone oxime. In addition, repeated dose studies with both oximes yielded similar patterns of haematotoxicity at similar dose levels. Hence, RAC considers the read-across from butanone oxime for the endpoint STOT SE as acceptable.

Regarding germ cell mutagenicity, read-across is needed for chromosomal mutations as no *in vitro* or *in vivo* cytogenicity studies are available for acetone oxime. RAC considers the available data on mutagenicity for the source and target substances comparable. *In vitro*, butanone oxime was concluded negative for the induction of bacterial and mammalian gene mutations, and no induction of chromosome aberrations and sister chromatid exchange (SCE) in Chinese Hamster ovary (CHO) cells or damage to DNA synthesis were observed in the Unscheduled DNA Synthesis (UDS) with rat hepatocytes. *In vivo*, negative results were seen in both the chromosome aberration assay in the bone marrow of rats (single dose) and the micronucleus test in peripheral blood erythrocytes in B6C3F1 mice. In DNA extracted from the liver of rats exposed to butanone oxime once via inhalation, DNA adducts were not observed. In comparison, studies with acetone oxime were also reported negative for *in vitro* bacterial and mammalian gene mutations. Additional non-standard *in vitro* studies were negative including DNA repair in UDS assays in V79 cell lines, ovine seminal vesicles (OSV) cells and rat primary hepatocytes, and DNA strand breaks based on a negative *in vitro* alkaline comet assay. Both substances were reported to produce RNA adducts. In the case of acetone oxime also oxidative DNA modifications in rats (*in vivo*) were shown in non-GLP studies. Overall, the available data on acetone oxime and the more data rich source substance butanone oxime are comparable and not contradicting. RAC considers the read-across from butanone oxime acceptable to classify acetone oxime for germ cell mutagenicity. However, uncertainties due to formation of the genotoxic hepatocarcinogen 2-nitropropane (2-NP) upon acetone oxime oxidative metabolism and tautomeric equilibrium following propane-2-nitronate (P2-N) formation are noted. P2-N and 2-NP were shown to be formed *in vitro*, but 2-NP has been detected only in small amounts in *in vitro* liver microsome studies. In an *in vivo*

study, acetone oxime and P2-N were excreted in urine in comparable but small amounts. In this study, however, no other metabolites are reported and no information on excretion in faeces was reported. It is thus unclear to RAC whether 2-NP is actually formed in significant amounts *in vivo* presenting a potential mutagenic concern, especially as acetone oxime is also generated as a product of the metabolic detoxification (reduction) of the P2-N and 2-NP. 2-NP itself is positive in the Ames test, however acetone oxime and its related source substances were all negative for bacterial gene mutation. The difference in the metabolic pathways (i.e. conversion of butanone to the respective butanone nitronate) remains unclear, but RAC notes that uncertainties are reduced as Wasox-VMAC2 and Wasox-MMAC2 are also considered for read-across for mutagenicity and these quickly hydrolyse to acetone oxime and the respective silanol (see further below on read-across to Wasox-VMAC2 and Wasox-MMAC2).

As regards to liver carcinogenicity and read-across to butanone oxime, RAC notes uncertainties in the read-across. An early onset of carcinogenicity was suggested for acetone oxime based on the observed liver cell foci interpreted as pre-neoplastic lesion and seen at day 45 and sub-chronic (90 days) oral exposure to acetone oxime (see also section on carcinogenicity). No pre-neoplastic lesions were observed in any subacute and sub-chronic studies with butanone oxime and the available data is indicative of a rather late onset of tumour development due to butanone oxime exposure: Basophilic liver foci were only seen in the liver of male rats and only after 18 months of inhalation exposure to butanone oxime. Tumours were noted at $\geqslant$24 months in rats. In mice, liver changes indicating hepatotoxicity (necrosis, centrilobular, hepatocellular hypertrophy and granulomatous inflammation) were observed after $\geqslant$ 12 months of inhalation exposure to butanone oxime. Tumours in the mouse liver were detected after 18 months at study termination.
The reason for a difference in the onset of liver carcinogenicity is unclear, but the data may not be in contradiction to a read-across justification from butanone oxime to acetone oxime. Differences in the liver toxicity profile may be due to the different metabolite profiles of the two substances (e.g. due to formation of the hepatocarcinogen 2-NP following acetone oxime administration, see also section on toxicokinetics), however may also be due to differences in alkyl chain lengths or other so far unknown reasons, eventually leading to higher carcinogenic potency of acetone oxime. It is acknowledged that in sub-chronic studies with other simple aliphatic oximes (e.g. 2-pentanone oxime or cyclohexanone oxime), liver cell foci were also not detected (see ECHA dissemination site, recent RMOA on oximes by the DE CA and PC comment). Nevertheless, it cannot be ignored that liver lesions were consistently observed in all three available studies with acetone oxime (although 2/3 studies have to be considered insufficiently reliable for classification). Thus, it also cannot be excluded that acetone oxime may be a more potent liver toxicant and carcinogen with a considerably earlier onset of neoplastic liver lesions when compared to butanone oxime. Unfortunately, no chronic toxicity/carcinogenicity data on other simple aliphatic oximes are available to further assess this issue and to further support the read-across for liver carcinogenicity attributed to the common functional group.

Overall, RAC acknowledges the uncertainties with respect to the proposed read-across for the hazard class carcinogenicity. However these uncertainties do not indicate that acetone oxime does not pose a hepatocarcinogenic hazard, but rather add to the identified concern indicating a higher carcinogenic potency with a consistent pattern of liver effects in the acetone oxime data, which may be attributable to differences in chain lengths and/or metabolic profiles.

The read-across approach from butanone oxime seems plausible for the other two hazard classes, STOT SE and Mutagenicity.

Wasox-MMAC2 and Wasox-VMAC2 were specifically considered only for read-across on germ cell mutagenicity. These two substances release two or three moles of acetone oxime and one mole

of reactive methyl or vinyl substituted silanetriol after rapid abiotic transformation. The read-across from Wasox-VMAC2 and Wasox-MMAC2 seems plausible due to the quick hydrolysis of the parent compounds to acetone oxime and the respective silanol, which was suggested to quickly condensate with other silanols forming large siloxanes. The DS assumed that these condensation products are too large to become bioavailable. Uncertainty, however, was noted with respect to these statements, as no evidence was provided demonstrating that i) the condensation of the silanes is rapid and complete, meaning that only biologically unavailable higher molecular weight siloxanes are formed and no oligomers and maybe, also uncondensed silanes remain, and ii) the silanes and their condensation products do not elicit any toxicity themselves (toxicity data is not available for any of these substances). This lack of (toxicity) data, increases the uncertainty for read-across from these acetone oxime-releasing silanes to acetone oxime.
Furthermore, no data was provided in the dossier demonstrating that the hydrolysis of the parent compounds (Wasox-VMAC2 and Wasox-MMAC2) is complete. RAC notes, however, that the hydrolysis half-lives for both source substances were reported to be < 1 h. In closer inspection of the available data (ECHA dissemination site and recent RMOA on oximes by the DE CA), it becomes clear that hydrolysis may in fact be much faster. In the available study the first measurement was conducted only after 45 minutes and at this time point between 55 – 90 % of the substance was hydrolysed (regardless of the pH). In addition, data on other, similar oxime-releasing, silanes such as the butanone oxime releaser 2-Butanone-O,O',O''-(phenylsilylidyne)trioxime (OS-9000; hydrolysis half-life < 5 min) or the 2-pentanone oxime-releaser 2-pentanone,O,O',O''-(methylsilylidyne) trioxime (OS 1600, hydrolysis half-life < 4 min), indicate that hydrolysis half-lives for Wasox-VMAC2 and Wasox-MMAC2 may in fact rather be in the range of a few minutes (ECHA dissemination site and recent RMOA on oximes by the DE CA). These additional data decrease the uncertainties regarding the rate of hydrolysis of the silanes and the fast release of acetone oxime.

As cytogenicity data are not available for acetone oxime itself, read-across from the source substances, Wasox-VMAC2 and Wasox-MMAC2, was specifically proposed by the DS for the hazard class Mutagenicity to add further weight to the assessment. It would be expected that cytogenicity of acetone oxime would be detected in studies on Wasox-MMAC2 and Wasox-VMAC2, due to the quick release of acetone oxime following hydrolysis. In line with the data on acetone oxime also these substances tested negative for mutagenicity in the Ames test. The read-across is therefore justified in the view of RAC. It is noted that with respect to mammalian toxicity, repeated dose toxicity studies with acetone oxime and the two silanes, all yielded similar patterns of haematotoxicity at similar dose levels. The DS, however, noted that based on experimental data, testicular toxicity has been attributed to the methyl/vinyl silane portion of the two analogue substances and inhibition of acetylcholinesterase activity was also found to be associated to the stable silanetriols. RAC therefore agrees that the data is only to be used to support the assessment of acetone oxime for germ cell mutagenicity. Overall, RAC considers the read-across from Wasox-VAC2 and Wasox-MMAC2 to acetone oxime for the endpoint mutagenicity as acceptable.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute dermal toxicity

The DS proposed classification as Acute Tox. 4, H312 based on three acute dermal toxicity studies and the lowest LD_{50} obtained for rabbits of > 1000 mg/kg bw and < 2000 mg/kg bw. An ATE of 1100 mg/kg bw has been assigned.

Comments received during consultation

Two MSCA supported the classification proposal.

Assessment and comparison with the classification criteria

Three dermal acute toxicity studies were presented by the DS, two studies in rats and one study with rabbits.

***Table 3**: Acute dermal toxicity studies for acetone oxime*

Study	Test substance and dose levels	Results
Unpublished study report (2012a) OECD TG 402, GLP, Klimisch 1 CRL:(WI) rats, m/f, 5/sex	Acetone oxime (99.6%) in water Limit test 2000 mg/kg bw, Contact time 24 h, test duration 14 days	**LD_{50} > 2000 mg/kg**
Unpublished study report (1989a) Supportive, Klimisch 3 Rat, m/f, 1/sex per dose	Acetone oxime in water (purity not stated) 100, 300, 1000 mg/kg bw Contact time 24 h, test duration 14 days	**LD_{50} > 1000 mg/kg**
Unpublished study report (1991b) Similar OECD TG 402, GLP, Klimisch 2 New Zealand White rabbit Range finder: 1/sex per dose Main test: 5/sex per dose	Acetone oxime in water (purity not stated) Range finder: 1000, 2000 mg/kg bw Main test: 0, 100, 500, 1000 mg/kg bw Contact time 24 h occlusive, test duration 15 days	Range finder: **LD_{50} < 2000 mg/kg bw** 1000 mg/kg bw: 1/2 dead animals 2000 mg/kg bw: 2/2 dead animals Main test: **LD_{50} > 1000 mg/kg bw** 1000 mg/kg bw: no mortalities

Two of these studies were considered well reported and reliable key studies by the DS. In an OECD TG 402 guideline compliant study in rats, the LD_{50} was > 2000 mg/kg bw based on a limit test. In a GLP study in rabbits, performed similar to OECD TG 402, the LD_{50} obtained was > 1000 mg/kg bw based on the main test and < 2000 mg/kg bw based on the range finding test. For this study the purity of the test substance was unknown, and the study was rated Klimisch 2. As compared to the range finder, dose levels were reduced to ≤ 1000 mg/kg bw in the main study and no mortalities were reported.

In a supportive study in rats, rated Klimisch 3 by the DS, the LD_{50} was > 1000 mg/kg after dosing with up to 1000 mg/kg bw. For this study no information is available on the rat strain and the test substance purity, in addition, the methodological details are incomplete. The study, nevertheless, supports the results reported above with a LD_{50} of > 1000 mg/kg bw.

In comparison to rats, rabbits were more sensitive in acute dermal toxicity studies with acetone oxime. It appears plausible, as also in the case of butanone oxime species differences were noted;

here, rabbit dams were more sensitive regarding acute oral toxicity (no acute dermal studies available on rats for comparison; acute oral LD_{50}: 930 mg/kg bw in male rats; estimated oral ATE value in pregnant rabbits: ≤ 160 mg/kg bw).

According to the criteria, Acute Tox 4 (dermal) should be assigned if the LD_{50}/ATE values are > 1000 and ≤ 2000 mg/kg bw. In agreement with the DS, RAC recommends **classification as Acute Tox. 4, H312** based on the lowest LD_{50} value of > 1000 mg/kg bw but < 2000 mg/kg bw in rabbits.

As an exact ATE cannot be determined based on the available data, a converted acute toxicity point estimate (cATpE) is to be used. The LD_{50} determined falls in category 4 for acute dermal toxicity: 1000 < ATE ≤ 2000 mg/kg bw. According to the CLP Regulation Table 3.1.2, this corresponds to a cATpE of 1100 mg/kg bw. RAC, hence, agrees with the DS proposed **ATE of 1100 mg/kg bw** based on conversion from experimentally obtained acute toxicity range values.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Animal data indicated ataxia in rats after oral administration of a high dose of acetone oxime in an oral acute toxicity study. Hypoactivity and lethargy were also described in rabbit and rat acute dermal toxicity studies; however, the findings in rabbits were considered to be possibly confounded by general systemic toxicity. Overall, the DS concluded that these observed transient clinical effects are indicative of narcosis.

Although the evidence from the animal data is not considered fully comprehensive, the chemical structure of low molecular oximes was suggested to be indicative of narcotic effects, in general. Derelanko and Rusch (2008) analysed structure/toxicity relationships of oxime silanes and reported that narcosis has been found consistently with low molecular oximes such as acetone oxime, but data on acetone oxime *per se* are unpublished according to the study authors.

The analogue substance butanone oxime met the classification for STOT SE 3, H336 (May cause drowsiness or dizziness) according to RAC (2018). Due to the structural similarity of butanone oxime and acetone oxime, including the common functional oxime group, the DS considered STOT SE (H336) classification for acetone oxime justified as well. The DS noted that the effect levels of acetone oxime with respect to narcosis may be higher compared to that of butanone oxime.

Overall, the DS considered that there is sufficient evidence for acetone oxime eliciting transient narcotic effects based on decreased activity, ataxia and/or lethargy in laboratory animals observed after single exposure and based on read-across with supporting information on the structurally similar butanone oxime. The DS concluded acetone oxime meets the criteria for classification as STOT SE 3, H336 (May cause drowsiness or dizziness) according to Regulation (EC) No. 1272/2008.

Comments received during consultation

Two MS submitted comments with respect to this hazard class. Both MS agreed with the proposed classification.

Assessment and comparison with the classification criteria

There are three acute toxicity studies available with acetone oxime and several additional studies testing the structurally similar substance, butanone oxime. In all three acute toxicity studies with acetone oxime, transient and reversible clinical signs were detected resembling narcosis.

In an acute oral toxicity study with acetone oxime (OECD TG 401, GLP compliant, Klimisch 2 assigned by the DS, purity of test substance not reported), five male and five female rats were treated with acetone oxime at a dose of 300, 1000 and 3000 mg/kg bw. One male that received 3000 mg/kg died two days after dosing. Dose-related reductions in body weight and body weight gain were reported and did not recover at ≥1000 mg/kg bw. Ataxia ("3 animals") and hypoactivity ("several animals") were reported as treatment-related effects immediately after oral administration of 3000 mg/kg bw (hypoactivity was noted up to day 4 post-exposure). Ataxia and hypoactivity also occurred in one animal two hours after dosing with 1000 mg/kg bw. No neurological or clinical signs were observed at 300 mg/kg bw. Effects on the haematological system were also investigated in this study using satellite groups at 300 and 3000 mg/kg bw. Dose-related methaemoglobinemia and anaemia were reported, however, no indication of the magnitude was reported.

In an acute dermal toxicity study with acetone oxime in rabbits (similar to OECD TG 402, GLP, Klimisch 2 assigned by DS), five animals per sex were exposed to doses of 100, 500 and 1000 mg/kg bw. One animal died at the high dose. Hypoactivity and some additional clinical signs (faecal staining, dark iris colouration) were observed in several animals at 1000 mg/kg bw. Dose-related methaemoglobinaemia was reported on day 1 and anaemia on day 1 and 5, particularly at the high dose. Body weight and organ weights were not affected.

In a supportive acute dermal toxicity study with acetone oxime in rats (OECD TG 402, GLP not specified, Klimisch 3 assigned by DS), one male and one female were exposed to 100, 300 and 1000 mg/kg bw for 24 hours (additional observation period of 14 days). Despite the limited documentation, lethargy and a dose dependent decrease in male bw gain were reported in animals of all dose groups post-exposure.

The highest dose tested in a repeated dose toxicity study with acetone oxime was 250 mg/kg bw/d, and no clinical signs indicative of narcosis were reported (OECD TG 408, GLP, Klimisch 2, unpublished study report (1991c), for details see section on STOT RE).

No further relevant studies and no mechanistic information on acetone oxime are available for this endpoint. It is noted that the role of metabolism/hydrolysis and a contribution of the metabolite acetone might be possible with regards to the observed transient acute effects. However, in a publication by Derelanko and Rusch (2008) on the structure/toxicity relationships of oxime silanes, it is stated that narcosis can be found consistently with low molecular oximes such as acetone oxime. The relevant data on acetone oxime, however, were unfortunately unpublished according to the study authors, limiting a firm assessment and conclusion on this assumption for this substance.

In addition to the data available for acetone oxime *per se*, the DS performed a read-across from the structurally similar substance, butanone oxime, according to the ECHA guidance.

Read-across

The analogue substance butanone oxime meets the STOT-SE classification criteria, as it recently received a harmonised classification for STOT SE for its narcotic effects in rats and rabbits after acute oral, inhalation and dermal exposure (i.e. STOT SE 3, H336: May cause drowsiness or dizziness) (RAC, 2018).

The detailed read-across justification can be found in section "RAC general comment".

The key studies used for the read-across from butanone oxime for this hazard class have been analysed by the DS for adequacy and reliability (Klimisch scores of 1 or 2 assigned).

A single oral dose of ≥ 300 mg/kg bw butanone oxime (gavage) was shown to elicit transient and reversible changes in neurobehavioral function consistent with CNS depression (changes in gait and aerial righting reflex), but no evidence of cumulative neurotoxicity was detected (Schulze and Derelanko, 1993; Germany, 2014). Blood parameters were not reported for this study.

In an acute inhalation toxicity study a strong but transient effect indicative of narcosis occurred in both sexes at 4.83 mg/L/4 h during the exposure (TL2, 1984; Germany, 2017).

In addition, also after dermal exposure, transient narcotic effects were observed at a dose of 18 mg/kg bw (Germany, 2017). In this study, no clinical signs but methaemoglobineamia and splenic erythrophagocytosis were reported at the mid dose (185 mg/kg bw). No mortality was observed at the mid and low dose, while all animals died within 48 hours at the high dose of 1848 mg/kg bw.

After sub-chronic 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 bw/d (Schulze and Derelanko, 1993). In a developmental toxicity study with butanone oxime in rabbits (dams), decreased activity and wobbly gait occurred already at much lower dose levels (≥ 40 mg/kg bw/d; Derelanko *et al.* 2003, Germany, 2017), indicating differences in susceptibility between species. In the latter study, signs indicative of haemolytic anaemia were reported as well (i.e. dark red or reddish/green coloured urine, dark red contents in the urinary bladder, enlarged spleens and brown, discoloured lungs, pale liver). In the respective dose-range-finding study, increases in reticulocyte counts (up to +78% at 40 mg/kg bw/d at GD 13) and MetHb formation (up to 29% at 40 mg/kg bw/d at GD 13) were noted at ≥ 10 mg/kg bw/d. In the CLH Opinion, RAC noted that based on these latter findings, "it is difficult to ascertain whether these clinical signs were indicative of a temporary narcotic effect or signs of general toxicity due to impending death" (RAC, 2018).

Overall, RAC concluded that although "findings in acute toxicity studies were not described in great detail, but there was a consistency in those observations that were made, showing narcosis at sub-lethal concentrations". RAC considered these findings as sufficient evidence for classification of butanone oxime as STOT SE 3, H336.

No mode of action was described for the narcotic effects of butanone oxime in the substance evaluation conclusion by the DE CA (2014) or in the RAC Opinion on butanone oxime (2018). RAC notes that MEK, one metabolite of butanone oxime, has a harmonised classification for narcotic effects (STOT SE 3, H336) as well. In addition, narcosis was also observed after administration of another oxime by inhalation, acetaldehyde oxime, to Wistar rats (OECD, 2006).

Also for acetone oxime, no mechanistic information is available. RAC notes there is some uncertainty whether the observed ataxia and hypoactivity is due to narcosis or whether this effect may be related to methaemoglobinemia and haemolytic anaemia that may have resulted in functional hypoxia. In most studies no data on the magnitude of the haemolytic anaemia and the MetHb formation are available to further assess this.

Due to the structural similarities between the source and the target substances, including the common functional oxime group, the DS concluded that it is justified to consider the narcotic effects observed for butanone oxime also for acetone oxime, in addition to the experimental evidence on the target chemical itself. The DS noted, however, that the role of metabolism/hydrolysis and a contribution of the metabolite acetone regarding these effects might also be possible. RAC agrees with this assessment.

Conclusion on the classification of acetone oxime for narcotic effects and comparison with the classification criteria

According to Regulation (EC) No. 1272/2008 for the purpose of classification for specific target organ toxicity – single exposure, substances are allocated to STOT SE Cat. 3 (H336) when transient target organ effects are observed after treatment that 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.

No guidance values are provided for category 3, since this classification is primarily based on human data and, if available, animal data in a weight of evidence evaluation. No human data on acetone oxime are available for this endpoint. The CLP criteria for classifying substances as STOT SE 3 for narcotic effects specify that 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, they shall be considered to support classification for STOT SE 1 or 2.

Animal data with acetone oxime indicate that oral administration of acetone oxime to rats at a high dose of 3000 mg/kg bw yields ataxia. Hypoactivity and lethargy were also described in rabbits and rats at doses ≥1000 mg/kg bw. While the evidence from the animal data is not fully comprehensive, the chemical structure of low molecular oximes was reported to be indicative of narcotic effects in general (Derelanko and Rusch, 2008).

Further evidence comes from the analogue substance butanone oxime, which meets the classification criteria for STOT SE 3, H336 (May cause drowsiness or dizziness) according to RAC (2018). Overall, the read-across approach provided in the present dossier seems plausible due to the structural and toxicological similarities of butanone oxime and acetone oxime (including the common functional oxime group). One of the main metabolites of butanone oxime, MEK, has a harmonised classification for STOT SE 3, H336 as well. In (acute) studies with butanone oxime, some effects indicative of haemolytic anaemia were seen, but RAC classified the substance particularly for its transient narcotic effects after single dosing.

Overall, the available data for acetone oxime on this endpoint is limited and effect levels for narcosis might be higher compared to butanone oxime. Nevertheless, the available data are indicative of acetone oxime eliciting transient effects resembling narcosis, as evidenced by decreased activity, ataxia or lethargy in laboratory animals after single exposure. Acetone oxime and butanone oxime thus have a comparable toxicological profile. Uncertainties were identified regarding whether the ataxia and hypoactivity observed in the above mentioned studies are in fact due to narcosis or may be related to haemolytic anaemia and MetHb formation. While uncertainties are noted for the acetone oxime data, the read-across to butanone oxime supports classification for narcotic effects. RAC considers the read-across justified and taken into account the available information on this source substance, as well as taking into account the supportive data on acetone oxime itself, RAC concludes 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.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation based on a GLP study performed similar to OECD TG 405 in rabbits, in which mean individual scores from gradings at 24, 48 and 72 hours for erythema and oedema ranged from 0 to 2 and 0 to 1, respectively. Skin reactions were not delayed and effects were fully reversible after five days.

Comments received during consultation

Two MSCA supported that acetone oxime does not meet the classification criteria for skin irritation.

Assessment and comparison with the classification criteria

One skin irritation study in rabbits was presented in the CLH report.

Table 4: *Skin irritation study on acetone oxime*

<table>
<tr><th>Study</th><th>Test substance and dose levels</th><th>Results</th></tr>
<tr><td>Unpublished study report (1990a)
Similar OECD TG 404, GLP,
Klimisch 2
New Zealand White rabbits
6 animals (4 males & 2 females)</td><td>Acetone oxime
0.5 g solid and moistened with 0.5 ml physiological saline solution per site
24 hours occlusive skin application with gauze patch.</td><td>Slight irritating and fully reversible at day 5 in all 6 animals (DRAIZE scoring with values averaged over days 1, 2, and 3):
<table>
<tr><th>Animal No.</th><th>Erythema score (moistened)</th><th>Edema score (moistened)</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>
</td></tr>
</table>

Acetone oxime was found to cause only slight and reversible skin irritation effects when tested in 6 rabbits with exposure to 0.5 g acetone oxime/site and in addition with 0.5 g acetone oxime/site, moistened with physiological saline solution, for an exposure period of 24 hours (occlusive). The DS highlighted that also in the dermal acute toxicity studies summarised above no local effects indicative of irritating or corrosive properties were reported.
In comparison with Table 3.2.2 of the CLP Regulation, classification criteria for skin irritation category 2 are not met as mean scores from the 24, 48, 72 hours gradings were below a value of 2.3 for erythema or oedema, no delayed reactions were observed, and all effects were reversible within five days. RAC concludes on **no classification for skin irritation/corrosion**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification and labelling of acetone oxime for 'irreversible effects on the eye' Category 1 (Eye Dam. 1), H318, based on one study performed similar to OECD TG 405 in rabbits, showing persistent severe eye lesions (pannus and corneal ulceration).

Comments received during consultation

Two MSCA supported the classification proposal.

Assessment and comparison with the classification criteria

One study is presented in the CLH report for evaluation of eye damage/irritation.

Table 5: *Eye irritation/damage study on acetone oxime*

<table>
<tr><th>Study</th><th>Test substance and dose levels</th><th>Results</th></tr>
<tr><td>Unpublished study report (1990b)
Similar OECD TG 405, GLP, Klimisch 2
New Zealand White rabbits
6 animals (3 males & 3 females)</td><td>Acetone oxime
0.1 g solid or 0.1 ml
21 days duration
Assessment at 1 hour, daily base until day 4, day 7, 10, 14 and 21 post-treatment.</td><td>Irreversible effects on the eye based on corneal damage not reversible within 21 days. Conjunctival irritation and iridial changes or damage.
Day 1: 6/6 animals grade 3 discharge and grade 2 conjunctival redness and chemosis, conjunctival necrosis (until day 7), corneal opacity and/or corneal ulceration.

Day 3: Corneal opacity and iris score max. 3 and 2 in 1/6 animal, respectively. In addition:

During the observation period, iris score of 1 in 3/6 animals and 0 in 2/6. Corneal opacity score of 2 in 1/6 animal, 1 in 3/6 animals and 0 in 1/6 remaining animal.

Corneal ulceration score 4: 3/6 animals day 1, 1/6 animals day 2.

Pannus: 2/6 animals exhibited pannus until study termination (days 7 – 21, days 10 – 21)

Reversibility: Ocular irritation and effects were reversible except in 2/6 animals (one with corneal opacity, ulceration and pannus at day 21, the other animal with persistent pannus from day 10 to study termination).

Individual mean DRAIZE scores over day 1, 2, and 3:
<table>
<tr><th>Mean scores over 24/48/72h</th><th>Animal #1</th><th>Animal #2</th><th>Animal #3</th><th>Animal #4</th><th>Animal #5</th><th>Animal #6</th></tr>
<tr><td>Chemosis (1)</td><td>2</td><td>2</td><td>1.7</td><td>2</td><td>1.7</td><td>1.3</td></tr>
<tr><td>Conjunctivae score (2)</td><td>2.3</td><td>2.6</td><td>2</td><td>2.6</td><td>2</td><td>2</td></tr>
<tr><td>Iris score (1)</td><td>1</td><td>1</td><td>1</td><td>1.3</td><td>0</td><td>0</td></tr>
<tr><td>Cornea opacity (2)</td><td>1.3(1)</td><td>1 (5, 4, 3)</td><td>1 (2)</td><td>2 (3, 4)</td><td>1 (2)</td><td>0</td></tr>
</table>
(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

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.</td></tr>
</table>

Acetone oxime was found to induce irreversible effects on the eye not reversible within 21 days.

For a study carried out using six rabbits, classification for irreversible effects to the eye is based on:

(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*.

In addition, for category 1 the following observations should be considered: "…*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…"*.

The classification criteria for serious eye damage (b) corneal opacity ≥ 3 and/or iritis > 1.5 are not met as the mean Draize scores over three days were below these mean scores in all tested

animals. However, score 4 corneal ulceration was observed in 4/6 animals at days 1 or 2 and pannus was additionally observed in two of these animals. Ocular irritation and effects were not reversible in 2/6 animals (1/6 animal with corneal opacity, ulceration and pannus at day 21, 1/6 animals with pannus from day 10 to study termination). In agreement with the DS, based on these persistent eye lesions (corneal ulceration and pannus), RAC recommends **classification and labelling of acetone oxime for 'irreversible effects on the eye' Category 1 (Eye Dam. 1), H318**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

For the evaluation of skin sensitisation a Guinea pig maximisation test (GPMT), a mouse ear swelling test (MEST, supporting study) and a Local Lymph Node Assay (LLNA) were presented by the DS. The DS proposed classification and labelling of acetone oxime as Skin Sens., sub-category 1B (H317) based on ≥ 30% responding at > 1% intradermal induction dose in a GPMT. Acetone oxime showed no sensitising property in the LLNA and no positive reaction was seen in the supportive MEST study.

Comments received during consultation

Two MSCA supported the classification proposal.

Assessment and comparison with the classification criteria

The skin sensitising potential of acetone oxime was investigated in guinea pigs and mice. One reliable Klimisch 2 OECD TG 406 compliant guinea pig maximisation test (GPMT) and a reliable Klimisch 1 OECD TG 429 Local Lymph Node Assay (LLNA) are available and further, a non-standard Mouse ear swelling test (MEST) assigned a Klimisch 4 score by the DS.

Table 6: *Skin sensitization studies on acetone oxime*

Study	Test substance and dose levels	Results
Unpublished study report (1990c) Guinea Pig Maximisation Test OECD TG 406, Klimisch 2, GLP Dunkin-Hartley guinea pig 15 females/test and PC group; 5 females/NC group	Acetone oxime <u>Induction</u> Intradermal: 5% in distilled water Topical: 100% solid (moistened with 0.9% saline), occlusive (day 7). <u>Challenge</u> Epicutaneous: 100% solid (moistened with 0.9% saline), occlusive (day 21).	**Sensitising** 24h after challenge: 6/15 (40%) 48h after challenge: 5/15 (33%) NC: 0/5 PC: 15/15 (2,4-dinitrochlorobenzene) Each at 24 and 48 hours after challenge
Unpublished study report (2013) Local Lymph Node Assay OECD TG 429, Klimisch 1, GLP Mouse (CBA), 4 females/group	Acetone oxime (purity 99.6%) 50%, 25%, 10% w/w in vehicle Acetone: olive oil (AOO) (day 1, 2, 3). Dorsal surface of ear.	**Not sensitising** 50 (w/v) % in AOO: SI 1.3 25 (w/v) % in AOO: SI 1.7 10 (w/v) % in AOO: SI 1.6 NC (AOO): SI 1.0 PC (HCA 25% w/v in AOO): SI 10.7
Unpublished study report (1989b) Mouse ear swelling test	Acetone oxime <u>Induction</u>	**Not sensitising** No reaction in any dosed and NC animals.

Non guideline, Klimisch 4, Limited documentation Mouse Balb/c 10 females/group 5 females/NC No PC	Epicutaneous: 35% w/v in milli-RO water open (day 1, 2, 3, 4, 7). <u>Challenge</u> Epicutaneous: 17.5% w/v (day 14, 21), open. (Pretreatment of mice with FCA intradermally injected on day 0)	(Ear thickness measured 0, 24, 48 hours after application and > 20% increase considered positive)

Acetone oxime showed a skin sensitisation response of ≥ 30 % at > 1.0 % intradermal induction dose in the adjuvant type test method (GPMT), as a clear response in 40 % of treated animals (6/15) 24 hours after challenge. The result is considered reliable (positive and negative controls were included in the study). However, RAC notes that no information on test substance purity is available, and the study is rather old.

A more recent non-adjuvant LLNA study is available, in which acetone oxime tested negative for skin sensitisation. The LLNA study was conducted with concentrations up to the solubility limit of 50 % and stimulation indices were 1.7, 1.6 and 1.3 at 10 %, 25 %, and 50 %, respectively. The positive control behaved accordingly. According to CLP, a significant sensitising effect is defined by a stimulation index of ≥ 3. Therefore, under the conditions of this study, acetone oxime was not sensitising.

The negative MEST is not considered suitable for evaluation of acetone oxime due to deficiencies and reduced sensitivity of the assay. MEST is not one of the three recognised and officially accepted animal test methods for skin sensitisation defined by OECD Test Guidelines. In addition, the DS highlighted a range of deficiencies as regards to study design and reporting. RAC does not add any further weight to this result.

As regards to the contradictory results obtained with the adjuvant-type GPMT and non-adjuvant LLNA, the use of Freund's Complete Adjuvant and both intradermal and topical exposure used during the induction phase may explain the qualitative differences in the test results as indeed the GPMT was designed to maximise sensitivity.

The DS reminded that also for the related substance butanone oxime the non-adjuvant LLNA was negative while the adjuvant type GPMT was positive. RAC classified butanone oxime as Category 1 for skin sensitisation, H317, in 2018. The weight of evidence for butanone oxime however was stronger as two GPMT and one Bühler test were positive. Still, the classification of the related substance butanone oxime adds further weight to the available evidence for acetone oxime.

It was further pointed out by the DS that the hydrolysis products of acetone oxime at acidic, basic and neutral pH, were predicted to be acetone and hydroxylamine according to the Hydrolysis Simulator of the OECD Toolbox. Hydroxylamine is a known sensitiser classified as Skin Sens. 1, H317, according to Annex VI CLP. For butanone oxime pH dependency of hydrolysis indicates degradation can be expected to be fast under acidic conditions (pH 4), and significantly slower at neutral pH and stable under basic conditions. The pH level of the skin is in the acidic range. In the view of RAC these considerations add limited weight for classification and labelling of acetone oxime.

A substance may be classified as a skin sensitiser on the basis of a positive test result in one of the recognised animal tests (i.e. GPMT, LLNA, Bühler). Despite the negative result from the LLNA, the weight of evidence provided indicates that acetone oxime should be classified as a skin sensitiser. The GPMT providing a positive result indicates classification in category 1 based on ≥

30 % responding at > 1 % intradermal induction dose. Lower concentrations (<1 %) were not tested, the presence of effects at lower doses therefore has not been shown and sub-categorisation is therefore not applied by RAC. Supportive weight for classification is added by read-across to the related substance butanone oxime which was also positive in GPMT tests.
RAC concludes on classification and labelling of acetone oxime as **Skin Sens. 1, H317, May cause an allergic skin reaction.**

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Based on adverse effects on the haematopoietic system and associated organs, the DS proposed classification of acetone oxime for target organ toxicity after repeated exposure, i.e. STOT RE 2, H373 (blood system), according to Regulation (EC) No. 1272/2008.

A sub-chronic oral repeated dose study in rats (Klimisch 2, GLP) indicated that haemolytic anaemia 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, haemosiderin) and extramedullary haematopoiesis in spleen and liver. Effects on the blood relevant for classification were observed at doses of ≥ 50 mg/kg bw/d.

At the interim time point (after 45 days of exposure) treatment with acetone oxime yielded haemolytic anaemia with decreases in haemoglobin (Hb) levels and red blood cell (RBC) counts of -10.1% and -11%, respectively, in males and -13% and -14%, respectively, in females at 50 mg/kg bw/d. After 90 days of exposure, compensation mechanisms attenuated the magnitude of Hb reduction, resulting in a slight, but not statistically significant decrease in haemoglobin values at study termination (i.e., after 90 days). At and above the mid dose of 50 mg/kg bw/d and both evaluated time points (45 and 90 days), other erythrocyte parameters like MetHb, erythrocyte count, mean corpuscular volume and mean corpuscular haemoglobin (in males and females) and reticulocyte and platelet counts (in males) were significantly different from controls as well. No urinalysis was performed at any time point.

In addition, at ≥ 50 mg/kg bw/d statistically significant increases in absolute and relative spleen weights were observed in females at the interim sacrifice (day 45) and at study termination (day 90), and weight increases were not fully reversible within 30 days of recovery. Absolut and relative spleen weights were also higher in males at ≥ 50 mg/kg bw/d when compared to the controls, but effects were only statistically significant in the high dose group (250 mg/kg bw/d) at study termination (90 days).

Corresponding histopathological effects in the spleen were not reversible during recovery and included extramedullary haematopoiesis, and pigmentation (haemosiderosis) as well as congestion of the red pulp. Capsular fibrosis was observed in two animals at the high dose. Furthermore, slight to moderate haemosiderin accumulation, minimal to slight extramedullary haematopoiesis as well as clear cell foci were observed in the liver of male rats at ≥ 50 mg/kg bw/d.

This combination of effects on the hematopoietic system and associated organs demonstrates adverse haemolytic anaemia after sub-chronic exposure to 50 mg acetone oxime/kg bw/day and warrant classification as STOT RE 2, H373 (blood system).

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) based on similar findings in repeated dose toxicity studies.

Comments received during consultation

Two Member States commented on this endpoint. Both MSCA pointed out that data may be considered borderline with respect to severity of histopathological effects and the potential to compensate the blood effects but agreed with the proposed classification of acetone oxime as STOT RE 2, H373 (blood system). Agreement was mainly due to the facts that i) adverse effects were already observed at a dose below the guidance threshold for STOT RE 2 (i.e. at 50 mg/kg bw/d), and ii) similar haematological findings were observed with the analogue substance, butanone oxime, for which RAC agreed on the STOT RE 2, H373 (blood system), classification.

Assessment and comparison with the classification criteria

There is one sub-chronic repeated dose toxicity study available (Klimisch 2, GLP), in which male and female SD-rats were exposed to acetone oxime at 10, 50 and 250 mg/kg bw/d by gavage (vehicle: water). 5 animals/sex/dose were sacrificed after 45 days of exposure (interim sacrifice), 10 animals/sex/dose were sacrificed after 90 days of exposure (study termination) and the remaining 10 animals/sex/dose were sacrificed after a 30-day treatment-free recovery period (recovery animals).

Dose-related and statistically significant effects, indicative of haemolytic anaemia were observed starting at 10 mg/kg bw/d, and becoming more numerous and more severe at the higher doses. As a compensatory reaction to the haemolytic anaemia, i.e. 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. Urinalysis was not performed.

After 45 days of exposure

Administration of 10 mg acetone oxime/kg bw/d for 45 days resulted in a decrease in Hb of -9 % in females and Hb levels were significantly reduced in both sexes at 50 mg/kg bw/d (- 10.1 % in males and -13.3 % in females). Similarly, RBC counts were reduced dose-dependently reaching approximately -20 % at 50 mg/kg bw/d after 45 days of exposure (- 17.1 % in males and -20.6 % in females). At the highest dose of 250 mg/kg bw/d, which is above the guidance value for STOT RE classification (would be 200 mg/kg bw/d for 45-day treatment duration), Hb and RBC counts were reduced by approximately 20 % and 37 %, respectively, in females, and 11 % and 36 %, respectively, in males. Haematocrit (HCT) values were significantly reduced at ≥ 10 mg/kg bw/d in females and at ≥50 mg/kg bw/d in males. The effects on Hb levels, RBC counts and HCT are indicative of a substance-induced destruction of mature erythrocytes.

In addition, slight and dose-dependent methaemoglobinaemia was observed as well (at 50 mg/kg bw/d: 2.9 % in males and 1.8 % in females). MetHb formation suggests that the haptoglobin binding capacity in the blood of the exposed animals exceeded due to the observed RBC destruction, yielding free Hb in plasma, which is subsequently oxidised to MetHb.

Furthermore, reticulocyte (RET) counts were significantly elevated at ≥ 50 mg/kg bw/d. At the same time, abnormal erythrocytic morphology consistent with polychromia was reported and occasional Howell-Jolly bodies were observed. The increases in RET counts point towards an enhanced reticulocytosis in the bone marrow as adaptive response to the accelerated RBC loss. Platelet counts were significantly increased in males at ≥ 50 mg/kg bw/d. Howell-Jolly bodies are

a cytopathological finding of basophilic nuclear remnants (clusters of DNA) in circulating erythrocytes.

Histopathological findings concurrent with the affected blood parameters included minimal to slight extramedullary haematopoiesis in the liver and spleen (m/f) with increasing severity from 50 to 250 mg/kg bw/d. Dose-dependent increases in liver and spleen weights were reported, a statistically significant weight increase at a dose level relevant for classification was observed in the spleen of females at 50 mg/kg bw/d (+66.4 %). At the high dose of 250 mg/kg bw/d marked and statistically significant increases in relative spleen and liver weights were observed in both sexes.

Moreover, slight to moderate pigmentation of phagocytic cells in the liver (suggested to be haemosiderin accumulation in the Kupffer cells lining the hepatic sinusoids and phagocytic macrophages in the periportal areas), as well as clear cell foci were reported at ≥ 50 mg/kg bw/d. The hepatocellular changes were reported to be more severe in treated male rats than in female rats and specific evidence of hepatotoxicity was observed only in treated males. It is noted that the time point at which these liver effects were noted is not given in the dossier or on the ECHA dissemination website, making it difficult to assign the observed liver weight increases to these histopathological findings. In addition, the DS considered the liver cell foci to represent pre-neoplastic lesions and the mechanism for tumour (and thus foci) development (for butanone oxime) was considered to not be associated with haemolytic anaemia (RAC, 2018; see also section on carcinogenicity). The relevance of these liver foci for haematotoxicity is, thus, uncertain. Nevertheless, a relevance of this finding for STOT RE classification cannot entirely be excluded.

In addition to the above reported effects, congestion of the red pulp as well as pigmentation in spleen (suggested to reflect haemosiderosis) were noted at ≥ 50 mg/kg bw/d. Capsular fibrosis was overserved in two animals at 250 mg/kg bw/d. Again, neither the time point (interim, terminal or recovery animals) nor the magnitude of these histopathological alterations were specified in the dossier, making it difficult to assign e.g. the observed spleen weight increases to an increased haematopoiesis or to increased haemosiderin deposition, or both.

Table 7: *Selected haematology parameters as reported in the dossier (blue: statistically significant effects below the guidance value for STOT RE 2).*

Dose/time	**Met-haemoglobin (%)**	**Haemoglobin in g/dl (% rel. to controls)**	**Haemato-crit (%)**	**Erythrocyte count (RBC) in mil/µL (% rel. to controls)**	**Reticulo-cyte count (% RET)**	**Platelet count (100 T/µL)**
45 d, n=5, male						
vehicle	0.4	16.9	44	7.27	0.3	11.33
10 mg/kg	0.7	16.5 (-2.4%)	44	7.23 (-0.5%)	0.7	12.12
50 mg/kg	**2.9****	**15.2 (-10.1%)***	**40****	**6.03 (-17.1%)****	**2.2***	**15.35****
250 mg/kg	**6.9****	**15 (-11.3%)****	**35****	**4.66 (-35.9%)****	**34.4****	**15.35****
45 d, n=5, female						
vehicle	0.8	17.3	46	7.19	0.2	10.99
10 mg/kg	0.7	**15.7 (-9.2%)****	**42****	**6.65 (-7.5%)***	0.8	12.13
50 mg/kg	**1.8***	**15 (-13.3%)****	**39****	**5.71 (-20.6%)****	**2.4***	13.79
250 mg/kg	**5.5****	**14 (-19.1%)****	**34****	**4.51 (-37.3%)****	**38.6****	13.76
90 d, n=10, male						
vehicle	0.8	15.2	45	7.64	0.2	11.55
10 mg/kg	1.1	15.2 (±0%)	45	7.64 (±0%)	0.6	12.42

Dose/time	Met-haemoglobin (%)	Haemoglobin in g/dl (% rel. to controls)	Haemato-crit (%)	Erythrocyte count (RBC) in mil/µL (% rel. to controls)	Reticulo-cyte count (% RET)	Platelet count (100 T/µL)
50 mg/kg	**3.2****	15 (-1.4%)	44	**6.8 (-11.0%)****	**1.6****	**14.09***
250 mg/kg	**8****	14.6 (-3.9%)	**38****	**5.28 (-30.9%)****	**13.7****	**15.01****
90 d, n=10, female						
vehicle	0.6	15.3	45	7.25	1.6	12.05
10 mg/kg	0.9	15.9 (+3.9%)	48	7.51 (+3.6%)	0.6	10.91
50 mg/kg	**2.7****	14.2 (-7.2%)	42	**6.25 (-13.8%)****	1.8	11.2
250 mg/kg	**7.3****	14.9 (-2.6%)	**39****	**5.45 (-27.6%)****	**12.8****	13.82
Recovery, n=10, male						
vehicle	0.5	16.2	45	7.93	1.2	13.01
10 mg/kg	0.4	16.6 (+2.5%)	46	8.2 (+3.4%)	**0.3****	13.71
50 mg/kg	0.5	16.8 (+3.7%)	47	7.9 (-0.4%)	**0.3****	12.88
250 mg/kg	0.5	**18.3 (+13.0%)****	**51****	7.94 (+0.1%)	0.6	12.17
Recovery, n=10, female						
vehicle	0.4	16.5	47	7.66	1	13.01
10 mg/kg	0.5	16.2 (-1.8%)	46	7.52 (-1.8%)	**0.3****	13.71
50 mg/kg	0.6	**17.4 (+5.5%)***	48	7.81 (+2.0%)	**0.2****	12.88
250 mg/kg	0.6	**17.9 (+8.5%)****	**50****	7.6 (-0.8%)	**0.4***	12.17

*p<0.05; ** p<0.01

***Table 8**: Selected body weight, liver and spleen weight as reported in the dossier (blue: statistically significant effects below the guidance value for STOT RE 2)*

Dose/time	Body weight (g)	Liver weight [organ/bw*100] (% rel. to controls)	Spleen weight [organ/bw*1000] (% rel. to controls)
45 d, n=5, male			
vehicle	418	3.19	1.81
10 mg/kg	421	3.25 (+1.9%)	1.93 (+6.6%)
50 mg/kg	414	3.47 (+8.8%)	3.87 (+113.8%)
250 mg/kg	431	**4.33 (+35.7%)****	**7.75 (+328.2%)****
45 d, n=5, female			
vehicle	226	3.25	2.14
10 mg/kg	249	3.13 (-3.7%)	2.37 (+10.7%)
50 mg/kg	234	3.14 (-3.4%)	**3.56 (+66.4%)***
250 mg/kg	228	**3.74 (+15.1%)****	**8.37 (+291.1%)****
90 d, n=10, male			
vehicle	498	3.09	2.09
10 mg/kg	528	2.98 (-3.6%)	1.78 (-14.8%)
50 mg/kg	501	2 (-35.3%)	3.11 (+48.8%)
250 mg/kg	480	**3.59 (+16.2%)****	**7.55 (+261.2%)****
90 d, n=10, female			
vehicle	273	2.88	2.66
10 mg/kg	279	2.79 (-3.1%)	2.66 (±0%)

Dose/time	Body weight (g)	Liver weight [organ/bw*100] (% rel. to controls)	Spleen weight [organ/bw*1000] (% rel. to controls)
50 mg/kg	274	2.76 (-4.2%)	**3.66 (+37.6%)****
250 mg/kg	271	**3.38 (+17.4%)****	**6.9 (+159.4%)****
Recovery, n=10, male			
vehicle	574	2.84	1.54
10 mg/kg	580	2.87 (+1.1%)	1.54 (±0%)
50 mg/kg	564	2.93 (+3.2%)	1.54 (±0%)
250 mg/kg	553	**3.31 (+16.5%)****	**2.3 (+49.4%)****
Recovery, n=10, female			
vehicle	294	2.81	1.73
10 mg/kg	293	2.83 (+0.7%)	1.78 (+2.9%)
50 mg/kg	289	2.73 (-2.8%)	1.79 (+3.5%)
250 mg/kg	281	3.04 (+9.3%)	**2.39 (+38.2%)****

*p<0.05; ** p<0.01

After 90 days of exposure

After sub-chronic exposure to acetone oxime, Hb levels were only slightly and non-significantly reduced in treated rats when compared to the controls pointing towards the potential to compensate the clinical signs of anaemia at least in part. Reductions in RBC counts, on the other hand, were still significant in both sexes at ≥ 50 mg/kg bw/d (≥ 10% at 50 mg/kg bw/d). RET counts were significantly increased in males at ≥ 50 mg/kg bw/d and in females at the highest dose of 250 mg/kg bw/d and cytopathological changes in erythrocyte morphology were noted. HCT was only significantly reduced at the high dose of 250 mg/kg bw/d (both sexes), while platelet counts were significantly increased in males at ≥ 50 mg/kg bw/d. Other erythrocyte parameters like mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were reported to be significantly different from controls at 50 mg/kg bw/d (no further details reported).

In addition, MetHb formation was statistically significantly increased at ≥50 mg/kg bw/d in both sexes, yielding generally slightly higher MetHb levels than after 45 days of exposure to acetone oxime (up to 3.2% and 8% in males at 50 and 250 mg/kg bw/d, respectively).

Increased bilirubin levels in males and females at 250 mg/kg bw/d (m/f) after 45 and 90 days are consistent with the observed lysis of RBC, the break-down of the haeme group in haemoglobin and conversion to unconjugated bilirubin. When more unconjugated bilirubin is produced by the macrophages than the liver can handle (by conjugation and excretion into bile), unconjugated bilirubin builds up in blood, leading to high total bilirubin values.

In males, absolute and relative spleen weight was significantly increased only at the highest dose, while spleen weights (absolute and relative) were significantly elevated in females at ≥ 50 mg/kg bw/d (relative: +37.6% at 50 mg/kg bw/d). Increases in liver weight were only significant in females at 250 mg/kg bw/d and not observed in males.

Again, concurrent minimal to slight extramedullary haematopoiesis was noted in the liver and spleen (m/f) with increasing severity from 50 to 250 mg/kg bw/d.

Moreover, slight to moderate Kupffer cell accumulation, as well as cell foci in liver were reported at ≥ 50 mg/kg bw/d. The hepatocellular changes were reported to be generally more severe in treated male rats than in female rats and specific evidence of hepatotoxicity was observed only in treated males. Again, it is noted that the time point at which these liver effects were noted, is

given neither in the dossier nor on the ECHA dissemination website. Similarly, for the observed congestion of the red pulp as well as the haemosiderosis in spleen reported at ≥ 50 mg/kg bw/d the time point of occurrence and magnitude of effect were not provided in the dossier. It is reported, however, that the "histopathological changes in the liver and spleen were increased in a dose-related manner".

After 30 days of recovery

Following a recovery period of 30 days after sub-chronic exposure of rats to acetone oxime, reticulocytosis and, thus, RET counts were significantly decreased in all treatment groups. Moreover, Hb levels were increased in both sexes at the high-dose and in females also at 50 mg/kg bw/d. Both effects point towards adaptive mechanisms compensating the haemolytic anaemia. Statistically significant increases in spleen and liver weight were still apparent at 250 mg/kg bw/d (except for liver weight in females, which was higher but did not reach statistical significance). The dose-dependent histopathological changes in the liver and spleen were reported to be not reversible during the 30-day recovery period.

Comparison with CLP criteria

According to Regulation (EC) No. 1272/2008 substances eliciting target organ toxicity after repeated oral exposure can be allocated to one of the following two categories: STOT RE 1 with a guidance value of C ≤ 10 mg/kg bw/d and STOT RE 2 with a guidance value range of 10 < C ≤ 100 mg/kg bw/d. 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 exposures) 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. Substances are classified in category 2 for target organ toxicity (repeat exposures) 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.

According to Regulation (EC) No. 1272/2008, section 3.9.2.5.2., the criteria for haematotoxicity are 'any consistent and significant' adverse changes in haematology. Specifically, any consistent and significant adverse effect in clinical biochemistry or haematology, significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination are considered relevant. As an example, haematotoxicity is considered severe if a marked increase in haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. Hb reduction of $\geqslant$ 10%) is found in a subacute or sub-chronic study.

However, it is noted that in the ECHA 'Guidance on the Application of CLP Criteria' (ECHA, 2017), it is further mentioned that in the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as "*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*" (Annex I, 3.9.1.4).

Guidance values to assist sub-categorisation are provided in the Regulation and respective Guidance Documents. However, based on the available data and only related to the observed haematotoxicity, it is not entirely clear if an adjustment of threshold values for varying study durations using dose/exposure time extrapolations according to Haber's rule as proposed in the ECHA Guidance can be considered appropriate in the present case. Considering the nature of the adverse effects and the potential to compensate clinical signs of anaemia at least in part, the

levels of effective doses are less clearly linked to the duration of exposure. However, any adverse haemolytic effect observed at a dose of up to 10 mg/kg bw/d and 100 mg/kg bw/d, respectively, either in a 28-day or 90-day study, should be considered as relevant for classification as STOT RE 1 or 2.

A repeated dose study with acetone oxime in rats indicated that haemolytic anaemia was the main toxic effect as shown by decreased red blood cell parameters and increased breakdown products of Hb, increased pigmentation (indicated to consist of deposits of iron: haemosiderosis) and extramedullary haematopoiesis in spleen and liver. In addition, the DS reported that haemolytic anaemia is consistently found with lower molecular weight ketoximes according to Derelanko and Rusch (2008).

No human data are available and no significant adverse effects relevant for classification of acetone oxime were observed at ≤10 mg/kg bw/d in the available study. Thus, classification of acetone oxime as STOT RE 1 is considered unjustified.

Relevant effects observed in the available repeated dose toxicity study with acetone oxime at doses determinative for STOT RE 2 classification included the reduction of Hb ≥10% at 45 days in both sexes, and reductions in RBC counts of approx. -14% in females and -11% in males after 90 days of exposure to 50 mg acetone oxime/kg bw/d. In addition, exposure to acetone oxime yielded increased formation of MetHb, which did not result in lethality but is indicative of acetone oxime being a MetHb generating agent. MetHb generating agents leading to haemolysis and anaemia shall be classified accordingly either in STOT-SE or STOT-RE according to the CLP Guidance. RAC notes that Hb reductions were not as pronounced after 90 days of exposure compared to the reductions observed after 45 days of exposure. This is indicative of a potential to compensate the haemolytic anaemia, at least in part.

The blood effects were observed together with slight but statistically significant increases in haemosiderosis in the spleen accompanied by increased organ weight. In addition, slight to moderate haemosiderin accumulation in the liver as well as minimal to slight extramedullary haematopoiesis in liver and spleen were reported at ≥50 mg/kg bw/d as well. It is noted that the severity of pigmentation/haemosiderosis in spleen and liver is only of slight to moderate severity. The ECHA guidance, however, states that classification is only warranted if marked increases in haemosiderosis in the spleen, liver or kidney in combination with other changes, e.g. a reduction in Hb levels of ≥ 10% were observed in repeated dose toxicity studies. Therefore, the reported histopathological effects may be considered as borderline for classification with respect to severity. Nevertheless, it is highlighted that the effects in liver and spleen were recorded at a dose of 50 mg/kg bw/d, which is considerable below the guidance level for STOT RE 2 classification. It can therefore be expected that severity of effects may become more pronounced at higher doses that are still within the range for classification, i.e. > 50 mg/kg bw/d and ≤ 100 mg/kg bw/d.
Capsular fibrosis, observed in two animals at 250 mg/kg bw/d, may be secondary to increased haemosiderin deposition, however, no information is given whether capsular fibrosis were seen after 45 or 90 days. If a haemolytic substance induces one or more of the serious health effects listed as examples below within the critical range of doses, classification is warranted according to CLP Guidance, section 3.9.2.5.2. on haematotoxicity. It is, thus, sufficient for classification if only one of these criteria is fulfilled. Criteria *"(e) multifocal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity"* may be fulfilled, but this assumption can only be verified, if DS can add further details on the observed fibrosis (distribution, severity, incidences after 45 and/or 90 days of exposure).

Moreover, criteria *"(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination"* may also be fulfilled for males at 250 mg/kg bw/d, as

they showed slight proliferating bile ducts in close association with macrophages containing hemosiderin-like pigment in the portal areas of the liver (see Table 13) . Haemosiderin deposition at any severity grade and bile duct proliferation as a proliferative response are pathological findings for the liver.

Supporting information

On the analogue substance, butanone oxime, RAC[1] recently concluded that the substance warrants classification as STOT RE 2; H373 (May cause damage to the blood system through prolonged or repeated exposure) based on similar findings on the blood system in repeated dose toxicity and chronic studies. In addition, data from these studies and the repeated dose toxicity study with acetone oxime indicate that the determined effect values are in the same range for both substances, if exposure duration is taken into consideration (NOAEL/LOAEL: 10 mg/kg bw/d; see also Table 30 in Annex I of the dossier).

Conclusion

Considering the entirety of adverse effects on the blood and the additional secondary effects in spleen and liver after repeated exposure of rats to acetone oxime at 50 mg/kg bw/d, a dose that is considerably below the upper guidance level for classification (i.e. 100 mg/kg bw/d), and further taking into account the clear dose-response relationship for the numerous parameters related to haematotoxicity (Hb reduction already starting at doses as low as 10 mg/kg bw/d), RAC concludes that classification of acetone oxime as **STOT RE 2, H373 (blood system)** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity based on the weight of evidence considering available studies on acetone oxime and read-across to the three analogues butanone oxime, the vinyl substituted silane Wasox-VMAC2 and the methyl substituted silane Wasox-MMAC2.
In several studies, acetone oxime did not produce gene mutations in tests with prokaryotic and mammalian cells *in vitro*. The analogue substance butanone oxime did not show evidence for SCE and chromosome aberration induction in CHO cells. Wasox-VMAC2 induced numerical and structural chromosome aberrations consistent with multiple chromatid breaks, fragments or interchanges *in vitro* in human lymphocytes, while Wasox-MMAC2 did not induce structural chromosome aberrations under the same test conditions. Wasox-VMAC2, and thus its two hydrolysis products including acetone oxime did not induce micronuclei in a follow-up *in vivo* mammalian erythrocyte micronucleus test; however, target organ exposure was not demonstrated. In another *in vivo* study, the analogue butanone oxime 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.
Supportive studies concerning indirect evidence of DNA damage, but not direct evidence of mutagenicity, showed that acetone oxime induced neither DNA strand breaks in an *in vitro* Comet assay nor DNA damage in unscheduled DNA synthesis in *in vitro* studies. However, two non-

[1] https://echa.europa.eu/documents/10162/fa6a43c5-6b2a-4c20-023b-801846dd19e3

guideline *in vivo* studies reported that acetone oxime caused DNA and RNA adduct formation (main modification 8-hydroxyguanine) in liver of F344 and SD rats after i.p. or oral administration indicative of oxidative stress. However, it was taken into account that adduct formation does not necessarily lead to mutations.
In a weight of evidence assessment, the DS considered that classification for germ cell mutagenicity was not warranted.

Comments received during consultation

Two MSCA supported no classification of acetone oxime for germ cell mutagenicity.

Assessment and comparison with the classification criteria

Acetone oxime has been evaluated in a battery of genotoxicity studies comprising *in vitro* gene mutation assays in bacterial cells, *in vitro* gene mutation assays in mammalian cells, *in vitro* UDS assays, *in vitro* comet assay as well as *in vivo* DNA and RNA adduct formation and the Drosophila SMART assay. No *in vitro* or *in vivo* studies on chromosomal aberrations and micronucleus induction are available for acetone oxime.

Table 9 presents on overview on the standard guideline assays performed with the substance. Most studies have a certain degree of deficiencies. Two of five studies on bacterial and mammalian gene mutation were rated Klimisch 2, the remaining were considered supportive by the DS.

Table 9: *In vitro mutagenicity studies on acetone oxime*

Study	Test substance and dose levels	Results
In vitro gene mutation assay, bacterial reverse mutation test		
NTP (2002) Klimisch 2 Similar OECD TG 471, GLP *S. typhimurium TA 1535, TA 97, TA 98 and TA 100* Preincubation method	**Acetone Oxime** <u>Main test - S9</u>: 0, 100, 333, 1000, 3333, 10000 µg/plate. <u>Main test + S9</u>: 0, 100, 333, 1000, 3333, 10000 µg/plate.	**Negative +S9/-S9** Deviations or deficiencies: • Only 4 strains tested (TA102 or E.coli WP2 missing). • No purity information reported • OECD max. conc. of 5 mg/plate exceeded • No detailed report
Mirvish *et al.* (1998) Klimisch 3 Test method according to Maron and Ames (1983) *S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100* Spot and plate assay	**Acetone oxime** <u>Plate assay</u> • TA100 & *TA98* S9/+S9: 0.25 – 2.5 µg/plate • *TA100 & TA1535* + S9: 2 – 8 mg/plate <u>Spot assay</u> • *TA 1535*, *TA 1537*, *TA 1538*, *TA 98* and *TA 100* Test +/- S9	**Negative +S9/-S9** Deviations or deficiencies: • One strain (TA1538) different then OECD TG471 (TA102 or E.coli WP2 missing). • No purity reported • Not all test concentrations documented • No detailed study report • OECD max. conc. of 5 mg/plate exceeded (TA100, TA1535
Araki *et al.* (1986) Klimisch 3 *S. typhimurium TA 2637, TA 98 and TA 100, E. coli WP2 uvrA/pKM101* Preincubation methods	**Acetone oxime** <u>-/+ S9</u> no concentrations nor controls reported/included	**Negative +S9/-S9** Deviations or deficiencies: • no detailed study report available • no information on purity, • no information on controls

In vitro mammalian gene mutation assays		
Unpublished study report (2012b) Klimisch 2 Mouse Lymphoma assay OECD TG 490 /former OECD TG 476, GLP Mouse lymphoma L5178Y cells	**Acetone oxime** <u>3 hours treatment</u> • Test + S9: 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL • Test –S9: 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL <u>24h treatment:</u> • Test –S9, 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL Tests conducted duplicate PC –S9: 4-Nitroquinoline-N-oxide PC +S9: Cyclophosphamide Solvent (DMSO) and untreated controls	**Negative +S9/-S9** Deviations or deficiencies: • cytotoxicity was determined by relative survival instead relative total growth (RTG) as recommended by the OECD guideline • Acceptability criteria cloning efficiency for solvent and untreated control not met (<65%). • top dose selection not in line with new recommendations Acceptability criteria for 3 assays met.
Haas-Jobelius *et al.* (1991) Klimisch 3 Similar OECD TG 476 Chinese hamster lung fibroblasts (V79)	**Acetone oxime (purity 98%)** • Test -S9 0, 0.23, 0.45, 0.5 mM Top dose was chosen based on 20% RS (relative survival) Solvent DMSO (1% v/v) PC: Isopropyl hydroxylamine	**Negative -S9** Deviations or deficiencies: • 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

Acetone oxime tested negative in three bacterial mutagenicity studies in the presence and absence of metabolic activation. Each of the three studies show deficiencies, for instance, none of the three studies including the NTP study tested all strains as required by the guideline, information on purity information is not available for any of the studies, and all studies have limited reporting and documentation. Thus, none of the studies qualify for a Klimisch 2 score even if NTP conducted one of the studies (implying that the study can be considered reliable). All studies were reported to be unequivocally negative in presence or absence of metabolic activation. Taken together, the studies included all strains needed to fully detect the range of mutagens including cross-linking mutagens in TA102 or DNA repair-proficient strain of *E. coli*. However only Araki *et al.* (1986) tested *E. coli* WP2 uvrA/pKM101 but the study comes with considerable uncertainties as test concentrations are unknown and the test was conducted only in the absence of metabolic activation. This study therefore is only of limited reliability in the view of RAC.

Remaining uncertainties as regards to the gene mutation potential are further reduced because gene mutation potential was assessed also in studies using mammalian cells. One of them, the mouse lymphoma assay, was a guideline and GLP study (unpublished 2012b) rated Klimisch 2 by the DS. Here, acetone oxime tested negative in presence and absence of metabolic activation in the mouse lymphoma L5178Y cells. Although some deficiencies are noted as regards to the acceptability criteria of the control groups cloning efficiency, the trials conducted in duplicate for 3 hours -S9 and + S9 and 24 hours -S9 were negative and proper controls were included, the acceptability criteria for the three assays were overall met. The second study reported by Haas-Jobelius (1991) in Chinese hamster lung fibroblasts (V79) seem to have significant deficiencies in study design and reporting and thus is of limited value in the view of RAC.

No adequate tests with acetone oxime for structural chromosome aberrations/clastogenicity were available. Therefore, read-across information from the related substances butanone oxime and the two silanes was considered by the DS. Upon hydrolysis of the silanes Wasox-MMAC2 and Wasox-VMAC2 acetone oxime will be released. The mutagenic potential of the structurally similar butanone oxime has been studied in a series of standard and modified *in vitro* and *in vivo* tests. RAC (2018) considered butanone oxime non-genotoxic and proposed no classification for germ cell mutagenicity.

The detailed read-across justification can be found in the section "RAC general comment".

***Table 10**: In vitro mutagenicity studies on butanone oxime, Wasox-MMAC2 and Wasox-VMAC2*

Study	Test substance and dose levels	Results
Read-across (see RAC 2018) NTP (1999) *In vitro* chromosome aberration Similar OECD TG 473 Chinese Ovary Hamster cells	**Butanone oxime (99.5%)** Up to 5000 µg/l +/- S9	**Negative +/- S9** Deviations or deficiencies • Only up to 200 instead of 300 metaphase cells analysed
Read-across Unpublished report (2005a) *In vitro* chromosome aberration OECD TG 473 Klimisch 2 Primary human lymphocytes	**Wasox-MMAC2** <u>3 hours treatment:</u> • - S9: 5.000, 1.670, 0.560, 0.185 µL/mL • + S9 (5%) 5.000, 1.670, 0.560, 0.185 µL/mL <u>20 hours treatment:</u> • -S9: 5.000, 1.670, 0.560, 0.185 µL/mL <u>Controls:</u> Methylmethanesulfonate –S9, Cyclophosphamide +S9	**Negative +/- S9** -S9 20 hours trial: highest test substance concentration was not analysed for chromosome aberrations, Mitotic Index (MI) of 7%, due to a very high cytotoxicity (at 1670 µg/L MI of 45%) Deviations or deficiencies • 100 instead of 300 metaphases were investigated per • concentration • No report information whether also for the 3h incubation period 1.5 cell cycles occurred. • No information on by-products or impurities
Read-across Unpublished report (2005b) *In vitro* chromosome aberration OECD TG 473 Klimisch 2 Primary human lymphocytes	**Wasox-VMAC2** <u>3 hours treatment:</u> • - S9: 5.000, 1.670, 0.560, 0.185 µL/mL • + S9 (5%): 5.000, 1.670, 0.560, 0.185 µL/mL <u>20 hours treatment:</u> • -S9 / 5.000, 1.670, 0.560, 0.185 µL/mL <u>Controls:</u> Methylmethanesulfonate –S9, Cyclophosphamide +S9	**Positive 20 hours – S9** **Negative 3 hours +/- S9** -S9 20 hours trial: Two highest test substance concentration not analysed for The other doses caused test concentrations related numerical and structural chromosome aberrations (multiple chromatid breaks, fragments or interchanges). Deviations or deficiencies • 100 instead of 300 metaphases were investigated per • concentration • No report information whether also for the 3h incubation period 1.5 cell cycles occurred. • No information on by-products or impurities

In the NTP study, butanone oxime was negative for induction of SCE 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.

In a GLP conform *in vitro* chromosome aberration test with human lymphocytes the vinyl substituted silane (Wasox-VMAC2) was positive without metabolic activation for the 20-hour treatment, but negative for the 3-hour treatments +/- S9. The methyl substituted silane (Wasox-MMAC2) was negative under the same test conditions. Whether this difference is associated with the vinyl/methyl silane portion of Wasox-VMAC2 is unclear. The result will be evaluated in a weight-of evidence with the available *in vivo* data presented in the following paragraphs.

Two *in vivo* studies are available for the read-across substances, one *in vivo* follow-up Mammalian Bone Marrow cytogenicity study with Wasox-VMAC2 and an Erythrocytes Micronucleus assay with butanone oxime. *In vivo* studies are not available for acetone oxime and Wasox-MMAC2.

Table 11: *In vivo mutagenicity studies on butanone oxime, Wasox-MMAC2 and Wasox-VMAC2*

Study	**Test substance and dose levels**	**Results**
Read-across (see RAC 2018) Unpublished study report (1990d) Chromosomal aberration assay Similar EPA OPPTS 870.5385 (*In vivo* Mammalian Cytogenetic Tests: Bone Marrow Chromosomal Analysis) Klimisch 2 Rat (Sprague-Dawley) male/female, 5/sex/dose	**Butanone oxime** Dose levels 300, 600, 1200 mg/kg bw, single oral dose (gavage)	**Negative** No significant increase in chromosomal aberrations in the bone marrow Toxicity: yes; Vehicle and positive controls valid.
Read-across Unpublished study report (2007) Mammalian Erythrocyte Micronucleus Test, OECD TG 474 Klimisch 2 Mouse, strain Crl:NMRI BR 5 m/f per dose; high dose and control 10 m/f	**Wasox-VMAC2** 1000, 1500, 2000 mg/kg bw, single dose (dose volume uniformly 10 mL per kg body mass). Vehicle: corn oil Positive control: 40 mg/kg bw Cyclophosphamide	**Negative** No cytotoxicity in the bone marrow was noted (PCE/NCE ratio not effected) at 2000 mg/kg bw (highest dose tested according to guideline) Deviations or deficiencies • No information on by-products and impurities

The read-across substance Wasox-VMAC2 did not produce an increase 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 of oral administration. The negative result thus does not confirm the positive response obtained *in vitro* in the 20-hour trial and indicates that none of the two hydrolysis products including acetone oxime were positive in this system. However, no cytotoxicity in the bone marrow as proof of target organ exposure was shown, but the highest dose (Wasox-VMAC2) was tested according to guideline.

The *in vivo* chromosome aberration study with the analogue butanone oxime tested in male and female Sprague-Dawley rats did not show significantly increased chromosomal aberrations in the bone marrow after single oral doses by gavage of up to 1200 mg/kg bw.

RAC further adds that for butanone oxime another *in vivo* study was evaluated in 2018, an *in vivo* micronucleus test with B6C3F1 mice (5/sex/dose) administered butanone oxime in drinking water at doses of 0, 110/145, 200/340, 515/630, 755/1010 or 1330/3170 mg/kg bw/day for 13 weeks. At the highest dose tested, the population of circulating erythrocytes was markedly decreased and there was no increase in the frequency of micronucleated normochromatic erythrocytes observed in male or female mice at any exposure concentration.

RAC considered the negative results of the two butanone oxime *in vivo* tests consistent with the findings seen *in vitro,* both demonstrating that butanone oxime lacks the potential to damage chromosomes. Overall (see also general RAC comment on read-across), butanone oxime was concluded negative for bacterial and mammalian gene mutation, and no induction of chromosome aberrations and SCE in CHO cells or damage to DNA synthesis were observed in the UDS with rat hepatocytes. *In vivo*, negative results were seen in both the chromosome aberration assay in the bone marrow of rats and the micronucleus test in peripheral blood erythrocytes in B6C3F1 mice. In liver DNA from rats exposed to butanone oxime once via inhalation, DNA adducts were not observed.

Furthermore, the DS presented non-standard studies and indicator tests capable of detecting DNA lesions. These studies were only briefly reported in the CLH report and considered supportive by the DS (no Klimisch score was provided):

In vitro:

- 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). According to the DS the study followed partly the deleted OECD test guideline 482. These cell lines are capable of expressing sulfotransferases SULT1A1 and SULT1C1 and 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.
- Kreis *et al.* (2000) showed also that the compound did not induce DNA repair in V79 cell lines using similar concentration range up to 10 mM and 5 hours treatment.
- In ovine seminal vesicle cells that lack cytochrome P450 enzymes but express phenol sulfotransferase or in cultured rat hepatocytes, acetone oxime did not induce DNA repair or any detectable DNA modification (DX1, 8-aminodGuo, 8–oxodGuo) according to Kreis *et al.* (1998).
- Haas-Jobelius *et al.* (1991) found no induction of DNA repair (test protocol partly in line with OECD 482, full report not available) 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) controls, the test compound did not induce a statistically significant increase in tail intensity (unpublished study report, 2016; no GLP).

In vivo:

- 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. Liver DNA and RNA were analysed after 6 hours following administration. In summary, the main DNA and RNA modifications were 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanine; the DNA modification was around 3 times higher 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. 2-NP increased levels of oxidative DNA and RNA adducts as well.
- Guo *et al.* (1990) 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. According to the DS, 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.

- The DS referred to the German substance evaluation of butanone oxime (2014) reporting that 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.
- Ryskova *et. al.* (1997) investigated the genotoxic potential of acetone oxime up to 5000 µM in the *in vivo* short-term 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). 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.

The oxidative DNA modifications 8-hydroxyguanosine and 8-hydroxy-2'-deoxyguanosine reported for acetone oxime *in vivo* were suggested to be the result of reactive oxygen species being formed via the intermediate P2-N which is in tautomeric equilibrium with the genotoxic hepatocarcinogen 2-NP. The lack of oxidative DNA adducts following butanone oxime administration, which does not form 2-NP, seems consistent with this suggestion. The significance of these DNA modifications however, as pro-mutagenic or even pro-carcinogenic lesions is unclear.

Conclusion on classification and labelling for germ cell mutagenicity

No epidemiological studies are available for acetone oxime and thus no classification in Cat. 1A is warranted. There is also 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.
For the evaluation of germ cell mutagenicity data from acetone oxime, butanone oxime and two oxime-releasing silanes are considered.

Taking the available evidence on *in vitro* gene mutation together, no concern has been identified for the substance acetone oxime for both the induction of bacterial or mammalian gene mutations. The remaining uncertainties attributed to the reliability of each of the studies on its own are considered acceptable by RAC and the substance is considered negative as regards to gene mutation potential. Additional non-standard *in vitro* studies on acetone oxime do not raise concerns for DNA strand breaks or DNA repair, as negative results were found in UDS assays in V79 cell lines, OSV cells and rat primary hepatocytes, and a negative outcome in the *in vitro* alkaline comet assay is reported.

Mammalian cytogenicity was not investigated for acetone oxime but for three related substances, one closely related structural analogue (butanone oxime), and two silanes (expected to quickly hydrolyse to acetone oxime). Wasox-VMAC2 induced cytogenetic damage *in vitro*, which was not observed for the analogue silane Wasox-MMAC2 and which was also not confirmed in a follow-up *in vivo* cytogenicity assay. Butanone oxime is considered negative as regards to gene mutation and cytogenicity based on *in vitro* and *in vivo* data (RAC 2018).

The non-standard *in vivo* studies on acetone oxime suggest oxidative DNA and RNA modifications in rats and genotoxicity in the Drosophila SMART assay. The reliability of all the non-standard assays presented by the DS is unclear and none of the studies was conducted according to validated guidelines. None of the rat *in vivo* studies investigated mutations but instead reported oxidative DNA modifications of unclear relevance to mutations.

In a weight of evidence assessment of the available standard *in vitro* and *in vivo* assay results on acetone oxime and the three related substances, acetone oxime is unlikely to have

genotoxic/mutagenic properties. **No classification of acetone oxime for germ cell mutagenicity is warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

To assess the carcinogenicity of acetone oxime no guideline and GLP compliant carcinogenicity study was available and the DS based the proposal for classification of acetone oxime as a Carc. 1B mainly on read-across from reliable experimental carcinogenicity studies with the analogue substance, butanone oxime. According to RAC (2018) and Annex VI CLP, butanone oxime has been assigned a harmonised classification as Carc. 1B; H350 (May cause cancer) with the general concentration limit of 0.1%.

Supportive experimental evidence for acetone oxime itself comes from a 90-day repeated dose study in SD rats, in which an early and 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 addition, an 18-months chronic study yielded hepatocellular adenomas in male MRC-Wistar rats after administration of acetone oxime via drinking water (Mirvish *et al.*, 1982). Moreover, in a HLN-assay in rats a significantly higher frequency of hyperplastic liver nodules compared to controls were detected (Mirvish *et al.*, 1988).

The mode of action for carcinogenicity is neither established for acetone oxime nor for butanone oxime. However, the DS assumed that based on the available mechanistic investigations and toxicokinetic information, metabolic activation to reactive intermediates and radical formation might play a role. For butanone oxime, RAC (2018) stated that it is unlikely that blood toxicity was a factor in hepatocarcinogenicity and based on limited evidence, a mode of action (MoA) that involves cytotoxicity was suggested. No other specific mechanism has been identified, thus, the observed tumours were considered as relevant to humans (RAC, 2018).

Also, a QSAR prediction gave a structural alert for carcinogenicity.

In a weight of evidence approach, the DS concluded that there is sufficient evidence to demonstrate animal carcinogenicity of acetone oxime and proposed classification as presumed human carcinogen, Category 1B, H350 (May cause cancer). The DS further proposed the general concentration limit of 0.1% to apply to acetone oxime, as it was considered not adequate to derive a T25 from the limited data on acetone oxime and from the read-across data on butanone oxime, respectively.

Comments received during consultation

Two MS commented on this endpoint and one comment was received from the Industry. The two MS agreed with the proposed classification of acetone oxime as Carc. 1B, H350, based on the reported studies on acetone oxime per se and the read-across approach applied by the DS.

The third comment provided by Industry considered the evidence regarding the carcinogenicity of acetone oxime as circumstantial as it is composed of several different threads of evidence, each of which is individually limited, i.e. i) data read-across from another substance, ii) liver nodule data, iii) unreliable carcinogenicity study data and iv) structure activity alert, in detail:

i) The read-across of data from butanone oxime for this endpoint is not unreasonable, but – by definition – read-across data carry some degree of uncertainty. This uncertainty should

be reflected in the classification of the target substance, i.e. strong evidence regarding the source substance has to be considered as weaker evidence for the target substance.

ii) The liver nodule data from the 90-day study is certainly indicative of a carcinogenic concern; however, in the absence of a reliable carcinogenicity study there is no firm evidence that these nodules would progress to neoplasms.

iii) The available carcinogenicity study on acetone oxime is relatively old, has a non-standard design, and only one dose was employed. Documentation, moreover, is scarce. Additionally, the control groups were staggered, which therefore adds an additional layer of uncertainty to the data. Taken together this makes the study unreliable. Whilst we would not want to fully dismiss the concerns raised by this study, it cannot be given the same status as the much more robust carcinogenicity data on butanone oxime. A category 1B classification is an overly conservative interpretation of an unreliable study.

iv) The QSAR prediction from the QSAR Toolbox V3.3.5 for oximes provides no supporting mechanistic chemistry. The CLH dossier argues that the liver cell foci are pre-neoplastic and are hence indicators for carcinogenicity. So if the carcinogenicity alert is robust then it would be anticipated that all such simple aliphatic oximes should consistently demonstrate this liver pathology. However, 90-day sub-chronic studies on Pentanone oxime and Cyclohexanone oxime do not demonstrate this liver pathology (source; Dissemination database). This would seem to suggest that this alert from liver cell foci is rather weak and not simply or strongly applicable across all such related oximes.

In conclusion, the industrial manufacturer considered none of the individual lines of evidence on carcinogenicity as definitive or strong enough to justify a classification of Category 1B. Due to the identified uncertainties regarding the proposed read-across and the available data on acetone oxime, a classification of acetone oxime as Carc. 2 was proposed.

Assessment and comparison with the classification criteria

Standard guideline carcinogenicity studies with acetone oxime are not available. However, a concern regarding the carcinogenic potential of acetone oxime comes from the structurally related substance butanone oxime which has a harmonised classification as Carc. 1B, H350.

The DS mainly used data on the analogue substance for justifying classification of acetone oxime. Supportive information comes from a GLP-conform sub-chronic study with acetone oxime in rats (OECD TG 408, Klimisch 2) and two supportive literature studies with acetone oxime which, however, were assigned a Klimisch score of 3 (not reliable).

QSAR estimations indicated an endpoint-specific structural alert for carcinogenicity ("Oncologic primary classification C-Nitroso and Oxime Type") for both substances. This profiler, however, was developed solely to mimic the structural classes of known/potential carcinogens covered in the US EPA OncoLogic Cancer Expert System (version 7.0) for predicting carcinogenic potential of chemicals.

Read-across

Butanone oxime is used as source substance for read-across for this endpoint (key study), as it displays a high structural similarity to acetone oxime and is classified as Carc. 1B, H350.

The detailed read-across justification can be found in section "RAC general comment".

The carcinogenic potential of butanone oxime has been studied in two combined chronic toxicity/carcinogenicity studies in F344 rats and CD-1 mice, respectively (see also Table 19 in the BD).

The combined chronic toxicity/carcinogenicity studies in rats and mice (similar to OECD TG 453; GLP not specified; Klimisch 2) demonstrate that butanone oxime causes liver tumours (adenomas and carcinomas) in both species at all tested exposure concentrations (see ***Table 12***).

Table 12*: 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 Purity: 99.9% 0, 15, 75, 374 ppm equivalent to 54, 270, 1346 mg/m³ Inhalation: vapour, 6h/d, 5d/week Duration: 26 months, interim sacrifice at 3, 12 and 18 months	Positive: Liver tumours: Lowest exposure level causing a significant increase at 75 ppm (270 mg/m³) (RAC, 2018) males: liver carcinomas 0/50, 0/51, 1/51, 12/51; statistically significant at 374 ppm 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	Newton *et al.* (2001) Germany (2014) and RAC (2018) Klimisch 2 Key study
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/m³) for liver carcinoma Carcinomas in males at 374 ppm (1346 mg/m³); and adenomas in all test groups ≥ 15 ppm (≥ 54 mg/m³); 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, 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

Statistically significant increases in the incidence of liver adenomas were observed at 270 and 1346 mg/m³ in male rats. Liver carcinomas were seen in male rats and male mice at 1346 mg/m³. In females, increases in incidence of liver adenomas occurred at the mid and high doses in rats and mice, but increases were not statistically significant. In addition, the incidence of fibroadenomas in the mammary gland was significantly increased in male rats at 1346 mg/m³.

Overall, RAC (2018) concluded on butanone oxime:

"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".

No (species-specific) mode of action for butanone oxime carcinogenesis was identified based on the available data, but RAC (2018) concluded that there is some limited evidence that liver (cyto)toxicity may have been a factor in the liver cancer seen in rats and mice, while blood toxicity (i.e. haemolytic anaemia, see also section on STOT RE) was excluded as potential mode of action of tumour development.

Butanone oxime and acetone oxime can hydrolyse to butane and acetone, respectively, and possibly to the common metabolite hydroxylamine. In addition, 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.

RAC (2018) calculated a T25 value for butanone oxime of 35.4 mg/kg bw/d which allocates this substance to the medium potency group. Thus, no SCL was derived for butanone oxime. The DS noted that it is not adequate to derive a T25 value for acetone oxime from the read-across substance butanone oxime.

Supporting information from a guideline study with acetone oxime

Supporting data come from an OECD TG 408 study with acetone oxime, in which hepatocellular changes in SD-rats were observed (Table 13).

Table 13*: Summary the results of the 90-d repeated dose toxicity study with acetone oxime*.

Study/Method	**Results**	**Remarks/ Reference**
equivalent or similar to OECD Guideline 408 Rat (Sprague-Dawley) male/female 25/sex/dose	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 the interim sacrifice at day 45.	Unpublished study report (1991c) Klimisch 2 Key study GLP

Study/Method	Results	Remarks/ Reference
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	The clear cell foci, composed of hepatocytes with clear finely granular cytoplasm, varied considerable in size and sometimes coalesced to form large areas of alteration. 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 vacuolisation (characterised 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.	Dosing volume was not adjusted to the same volume for the different dose levels

Clear liver cell foci with slight to severe grading were present in almost all animals at 250 mg/kg bw/d. Minimal clear cell foci were also detected after 90 days of exposure at 50 mg/kg bw/d. The DS noted that such cell foci are generally of low incidence in SD rats.

Basophilic cell foci were detected at the high dose of 250 mg/kg bw/d as well.

Lesions were generally more frequently observed in male rats compared to females and the onset of the foci of cellular alterations was reported to be dose-dependent and early in the treatment period (already seen at day 45). According to the DS, this early onset indicates that these lesions are tumour pre-stages.

The DS indicated that foci of cellular alterations 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, as reported in Thoolen *et al.* (2010). In addition, these alterations have been designated to play a precursor role in the process of hepatocarcinogenesis as they represent a localised 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 show common histomorphological characteristics which might be indicative of a mutual presumptive role in the process of hepatocarcinogenesis. The authors concluded that these focal cellular alterations occur spontaneously in aged rats, but also are considered as precursor lesions to hepatocarcinogenesis (Thoolen *et al.* 2012). However, not all foci were reported to be related to carcinogens (Thoolen *et al.* 2010).

RAC notes that pre-neoplastic liver lesions were not observed in most of the repeated dose studies with butanone oxime, a substance for which numerous *in vivo* studies in rats, mice and rabbits are available (subacute, sub-chronic and chronic, 2-generation study, developmental toxicity studies; routes: gavage, drinking water, inhalation). All liver effects observed in the available subacute and sub-chronic studies were almost certainly assignable to the haemolytic anaemia elicited by the substance (RAC, 2018). In a subacute oral (28-day) and a sub-chronic (90-day) inhalation study with butanone oxime in rats and mice, respectively (similar to OECD TG 407, GLP: study report, 1995; OECD TG 413, GLP: study report, 1995; information obtained from the ECHA dissemination site), the substance did not induce any significant hepatic peroxisome proliferation or liver cell changes, but significant increases in glutathione levels (primarily reduced glutathione) were observed at ≥ 250 mg/kg bw/day. No additional liver effects were reported.

After 12 and 18 months of inhalation exposure to butanone oxime, granulomatous inflammation of the liver was observed in male (43 % affected versus 24 % in controls) and female mice (43 % affected versus 32 % in controls) alongside increases in haemosiderin accumulation and enhanced centrilobular hypertrophy. Slight increases in incidence of liver necrosis were reported in females at the top dose (1346 mg/m³).
In addition, chronic inhalation exposure of rats to butanone oxime (≥ 0.054 mg/L) demonstrated an increase in spongiosis hepatis in males after 26 months. RAC (2018) stated in this context: "According to the scientific literature, this is a distinct lesion that may be associated with certain forms of hepatic neoplasia". In this study, an increase in liver weight of +40 % was reported as well, and slight increases in the incidence of intracytoplasmic vacuoles were noted in both sexes at the high dose. There was also an increase in the incidence of basophilic foci in hepatocytes in males and females compared to controls, with dose-dependent increase in severity. These foci were not reported at the 18 months interim sacrifice but only at study end, i.e. after 26 months of exposure.
Based on these findings, RAC concluded that there is limited evidence to suggest a mode of action that involved cytotoxicity for the increased incidences of liver tumours observed in rats and mice. No other specific mechanism of action could be identified.

In conclusion, the observed liver cell foci suggesting pre-neoplastic lesions after subacute (45 days) and sub-chronic (90 days) exposure to acetone oxime were not observed in subacute and sub-chronic studies with butanone oxime, and basophilic liver cell foci were noted only after chronic exposure of rats (26 months). The liver tumours in the carcinogenicity studies with butanone oxime appeared rather late in the life of the animals, with no significant increase in tumour incidence or sign of pre-neoplastic liver cell foci at 12 months of exposure in mice and 18 months of exposure in rats. The lack of pre-neoplastic liver lesions in short-term studies with butanone oxime and the late onset of tumour development does however not contradict the read-across to acetone oxime. This difference in tumour onset in the view of RAC is likely attributable to a higher carcinogenic potency of acetone oxime, for different possible reasons, including toxic metabolites and alkyl chain length. The early onset of these preneoplastic lesions rather increases the concern in the light of the butanone oxime carcinogenicity.

Supporting information from non-guideline, non-GLP studies with acetone oxime

Mirvish *et al.* (1982) investigated the carcinogenic potential of acetone oxime according to a non-guideline, non-GLP study (***Table 14***). Acetone oxime was administered to male and female MRC Wistar rats at a dose of 1000 mg/L drinking water for 18 months. Animals were further observed (without exposure) until they died naturally or in moribund condition and were not sacrificed at a specific time point. Liver tumour incidence was significantly higher in males (80%) when compared to controls (0%), but effects were not significant in females (17%). All analysed 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.

The liver adenoma incidence of 80% is remarkable, however, coming along with remarkable uncertainties as well, in the view of RAC. The study has major deficiencies, only one dose group was tested, no information on the purity of the test substance is available, small group sizes of 15-16 animals were employed, and a non-concurrent control group was run with 8 months of delay. In addition, poor study documentation is noted. After 80 weeks 11/15 (73%) males survived, survival decreasing dramatically with 2/15 (13%) after 100 weeks of study duration, despite cessation of exposure after 18 months (72 weeks). RAC notes that also the non-concurrent control animals died rather early in this study (mortality > 50 % in males after 80 days; > 50 % in females after 100 days) which further questions the reliability of the study.

Still, the reported findings may indicate a concern regarding the carcinogenic properties of acetone oxime as the same target organ is affected as for the similar substance butanone oxime (notably also the carcinogenic nitronate 2-NP identified in acetone oxime metabolism affects the same target organ). But robust conclusions cannot be drawn from this study on its own due to insufficient reliability.

Additional supporting information comes from a study by Mirvish *et al.* (1988), in which acetone oxime was investigated in a hyperplastic liver nodule (HLN) assay in Wistar and MRC-Wistar rats. In this study, induction of HLN was achieved by using a system that included a single diethylnitrosamine (DEN) treatment followed by partial hepatectomy and acetone oxime treatment (at 1000 mg/L) via drinking water for 8 weeks. A significantly higher frequency of hyperplastic liver nodules than in controls (treated with only DEN) was noted in treated animals (Table 14). The study authors indicate that most liver carcinogens have given positive results in this kind of assays, but since the first step in this test is treatment with a genotoxic carcinogen (DEN), the test actually measures the ability to promote liver carcinogenesis (Mirvish *et al.* 1988).

***Table 14**: Summary table of non-guideline animal studies with 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 accumulated dose/rat: 7 g/male rat, 6.2 g/ female rat Study duration: 18 months	LOAEL (carcinogenicity): ≤1000 ppm Incidence of liver tumours (adenoma) 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 (of the liver tumour) was described. 2 males had in addition haemangiomas.	Mirvish *et al.* (1982) (study report) 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[1])
Rat liver foci model male MRC-Wistar and Wistar rats; up to 10 animals/strain Test material: acetone oxime 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	Significantly higher frequency of hyperplastic liver nodules (HLN) compared to control. Authors suggested that acetone oxime may be a liver growth promotor.	Mirvish *et al.* (1988) No GLP Klimisch 3 Supportive study Purity: not stated

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

Conclusion on classification and labelling for carcinogenicity

According to section 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 the following hazard categories:

- Category 1 (Known or presumed human carcinogens) for substances, for which clear evidence is available largely coming from human case studies and/or epidemiological data (Cat. 1A) or which is mainly coming from animal studies (Cat. 1B). 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) for substances, for which the basis of evidence is 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. 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, guideline- and GLP-compliant carcinogenicity studies were not available. Classification of the substance as a carcinogen is, thus, largely based on weight of evidence of all relevant information, including read-across.

In absence of robust and reliable experimental chronic carcinogenicity information for acetone oxime, results from the analogue substance butanone oxime are considered in a weight of evidence with supporting subacute and sub-chronic data on acetone oxime. In accordance with the RAC opinion (2018), butanone oxime received a harmonised classification as Carc 1B; H350 (May cause cancer) with a general concentration limit (GCL) of 0.1%.

For butanone oxime, RAC concluded that the observed tumours are relevant for humans, as they were observed above the generally established background rates for the tested strains in reliable chronic studies. In addition, the available toxicokinetic evidence in animals provides no clear reason to suspect that a different mode of action may occur in humans.
RAC considered the evidence supporting a multi-site response as "not strong", but malignant liver tumours were observed in two species (rats and mice), specifically in one sex (males). The sex-specific findings were considered indicative of a gender-specific mechanism of carcinogenic action, as in female rats, only slight increases in benign tumours were observed at the top dose in the absence of statistical significance. A genotoxic mechanism of tumour induction was excluded and RAC further considered that haematotoxicity is not the underlying mechanism for tumour development, as the observed haematological effects "do not appear to follow the pattern of increased tumours observed in male rats of the mid and top dose groups and male mice of the top dose group only". It was considered possible, however, that cytotoxicity may be involved in tumour formation and that females may be merely less sensitive than males to the effects of butanone oxime, but no species-specific mode of action could be identified and there were no clear differences between histopathology results in males and females. Overall, RAC stated that "it cannot be concluded with certainty that butanone is a sex-specific carcinogen, although female rats and mice were clearly less sensitive than males in the available studies". Evidence of progression to malignancy of liver tumours, on the other hand, was considered clear and no reduction in tumour latency was observed in the available inhalation studies with butanone oxime. Moreover, RAC excluded the possibility of excessive toxicity at the tested dose as a confounding factor, as neither treatment-related increase in mortality, reductions in body weight compared to controls nor general signs of excessive toxicity were reported.

Supportive experimental evidence that increases the concern for acetone oxime being a liver carcinogen as well comes from a 90-day repeated dose study in rats, in which an early and dose-dependent onset of liver lesions consistent with foci of cellular alteration (clear cell foci, basophilic cell foci) were observed. Thoolen *et al.* (2012) considered that this type of liver foci may represent pre-neoplastic lesions. Liver cell lesions were not observed in any of the numerous available subacute and sub-chronic studies with butanone oxime. Basophilic liver foci as well as liver tumours were only observed after long-term exposure (i.e. after 18 months in mice and 26 months in rats). While these findings may be considered as an uncertainty in the proposed read-across (the assumption of an "early onset" tumour development due to acetone oxime exposure contradicting the reported late onset of carcinogenesis of butanone oxime), RAC, overall, is of the view that the early onset and dose-dependent increase of liver foci increases the concern for liver carcinogenicity in the light of butanone oxime hepatocarcinogenicity. These liver cell foci may be relevant for liver tumour development and cannot be disregarded in the acetone oxime hazard assessment by RAC. Whether the different metabolite profiles of the two substances (the shorter alkyl chain of acetone oxime compared to butanone oxime) or another, so far unknown, factor may be the reason for the observed differences in liver toxicity, remains unclear.

In an older 18-months chronic study (Mirvish *et al.*, 1982), hepatocellular adenomas were induced in male MRC-Wistar rats after administration of acetone oxime via drinking water. RAC notes, however, that the study has major deficiencies (e.g. only one dose group applied, purity of the test substance not reported, small group sizes, non-concurrent control group, poor documentation). Hence, uncertainty with respect to the reliability of the reported findings is considered very high. RAC does not add much weight for classification based on this study but acknowledges the apparent consistency in affected target organs, hepatocytes as cell type from which tumour growth originated and predominantly affected male versus female animals. Less sensitivity was also observed for female rats and mice treated with butanone oxime.

In a HLN assay in rats a significantly higher frequency of hyperplastic liver nodules compared to controls were detected in acetone oxime treated males (Mirvish *et al.*, 1988) following initiation treatment with a genotoxic carcinogen (DEN); however, the relevance of this tumour promotion finding is uncertain as well, as the test design is neither validated nor standardised.

QSAR predictions indicated a structural alert for carcinogenicity for both, butanone oxime and acetone oxime. Nevertheless, the predictions do not provide any supporting mechanistic chemistry and a mode of action for carcinogenicity is established neither for butanone oxime nor for acetone oxime. The QSAR alert may in fact be rather weak and application across all related ketoximes has limitations. No chronic studies on other simple aliphatic oximes are available.

Liver (cyto)toxicity was assumed to play a role with respect to butanone oxime (RAC, 2018) and based on the available toxicokinetic information on acetone oxime, metabolic activation to reactive intermediates and radical formation may contribute to tumour development. However, no evidence for these assumptions is available.

RAC overall notes that liver lesions were consistently observed in male animals in all three available studies with acetone oxime, although 2 out of 3 studies have to be considered insufficiently reliable for classification on their own. Similarly, butanone oxime elicited liver lesions and liver tumours with a rather late onset mainly in male rats and mice in chronic studies. This supports the read-across approach as with both substances, a sex-specific mode of action for liver carcinogenicity is suggested. In the view of RAC, the consistent pattern of early liver cell foci and tumours reported for acetone oxime in subacute and sub-chronic studies increases the concern and acetone oxime may be simply more potent in eliciting liver toxicity and liver tumour development with a considerably earlier onset when compared to butanone oxime.

Classification of acetone oxime for this hazard class is clearly dependent on the evaluation of the applied read-across and whether this information can be considered sufficient for classification of acetone oxime as Carc. 1B or rather justifies a Carc. 2 classification.

The CLP Guidance document states with respect to non-testing data (3.6.2.3.4): "*The specific category depends on the category of the known carcinogen and the degree of confidence in the robustness of the read-across prediction. The category will not be higher than the chemical used to read-across from, but normally may be the same. However a lower category may be applied if the read-across highlights a possible carcinogenic hazard, and thus supports a classification, but there is uncertainty as to the robustness of the read-across prediction or there is evidence, for instance from mechanistic or other studies, that the chemical may be of lower concern for carcinogenicity*".

The read-across from butanone oxime classified as Carc. 1B is generally considered plausible and justified. There is no evidence that would suggest acetone oxime to not exert the hazard identified for the source substance butanone oxime, thus "no classification" is considered inappropriate. Uncertainties were noted as regards to the early onset of liver lesions observed for acetone oxime. These liver lesions were however consistently observed in all three available studies with acetone oxime and may be indicative of a higher carcinogenic potency than the source substance. In the view of RAC it therefore cannot be concluded that the target substance may be of lower concern for carcinogenicity.
In a weight of evidence approach, RAC considers that the various lines of evidence provided in the dossier overall provide sufficient evidence that acetone oxime has a carcinogenic potential relevant for humans. Based on the read-across to butanone oxime and supported by animal experiments with acetone oxime, RAC considers that there is sufficient evidence justifying **classification as Carc. 1B, H350 (May cause cancer).** The general concentration limit of 0.1% should apply and no SCL is indicated for acetone oxime.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).