Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

butanone oxime; ethyl methyl ketoxime; ethyl methyl ketone oxime

EC Number: 202-496-6
CAS Number: 96-29-7

CLH-O-0000001412-86-227/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name:

Butanone Oxime

EC Number: **202-496-6**

CAS Number: **96-29-7**

Index Number: **616-014-00-0**

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Version number: **2.0**

Date: **May 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BUTANONE OXIME; ETHYL METHYL KETOXIME; ETHYL METHYL KETONE OXIME

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Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***butanone oxime***
EC number:	***202-496-6***
CAS number:	***96-29-7***
Annex VI Index number:	***616-014-00-0***
Degree of purity:	**-**
Impurities:	**-**

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	Classification		SCL/ATE/M-Factor
Current entry in Annex VI, CLP Regulation	Acute Tox. 4* Skin Sens. 1 Eye Dam. 1 Carc. 2	H312 H317 H318 H351	
Current proposal for consideration by RAC	Acute Tox. 3 Acute Tox. 4 Skin Sens. 1B Eye Dam. 1 Carc. 1B STOT SE 3	H301 H312 H317 H318 H350 H336	Oral: ATE = 100 mg/kg bw[1] Dermal: ATE = 1848 mg/kg bw[2]
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3 Acute Tox. 4 Skin Sens. 1B Eye Dam. 1 Carc. 1B STOT SE 3	H301 H312 H317 H318 H350 H336	Oral: ATE = 100 mg/kg bw[1] Dermal: ATE = 1848 mg/kg bw[2]

[1] Converted acute toxicity point estimate from Table 3.1.2 of CLP
[2] LD_{50} value for the dermal route

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BUTANONE OXIME; ETHYL METHYL KETOXIME; ETHYL METHYL KETONE OXIME

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not assessed in this dossier
2.2.	Flammable gases	None		None	Not assessed in this dossier
2.3.	Flammable aerosols	None		None	Not assessed in this dossier
2.4.	Oxidising gases	None		None	Not assessed in this dossier
2.5.	Gases under pressure	None		None	Not assessed in this dossier
2.6.	Flammable liquids	None		None	Not assessed in this dossier
2.7.	Flammable solids	None		None	Not assessed in this dossier
2.8.	Self-reactive substances and mixtures	None		None	Not assessed in this dossier
2.9.	Pyrophoric liquids	None		None	Not assessed in this dossier
2.10.	Pyrophoric solids	None		None	Not assessed in this dossier
2.11.	Self-heating substances and mixtures	None		None	Not assessed in this dossier
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not assessed in this dossier
2.13.	Oxidising liquids	None		None	Not assessed in this dossier
2.14.	Oxidising solids	None		None	Not assessed in this dossier
2.15.	Organic peroxides	None		None	Not assessed in this dossier
2.16.	Substance and mixtures corrosive to metals	None		None	Not assessed in this dossier
3.1.	**Acute toxicity - oral**	**Acute Tox. 3**	ATE = 100 mg/kg bw[3]	**None**	**Harmonized classification proposed**
	Acute toxicity - dermal	**Acute Tox. 4**	ATE = 1848 mg/kg bw[4]	**Acute Tox. 4***	**Harmonized classification proposed**
	Acute toxicity - inhalation	None		None	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	None		None	Data conclusive but not sufficient for classification

3.3.	**Serious eye damage / eye irritation**	**Eye Dam. 1**		**Eye Dam. 1**	**Harmonized classification proposed**
3.4.	Respiratory sensitisation	None		None	Data lacking
3.4.	**Skin sensitisation**	**Skin Sens. 1B**		**Skin Sens. 1**	**Harmonized classification proposed**
3.5.	Germ cell mutagenicity	None		None	Data conclusive but not sufficient for classification
3.6.	**Carcinogenicity**	**Carc. 1B**		**Carc. 2**	**Harmonized classification proposed**
3.7.	Reproductive toxicity	None		None	Data conclusive but not sufficient for classification
3.8.	**Specific target organ toxicity –single exposure**	**STOT SE 3**		**None**	**Harmonized classification proposed**
3.9.	Specific target organ toxicity – repeated exposure	None		None	Data conclusive but not sufficient for classification
3.10.	Aspiration hazard	None		None	Not assessed in this dossier
4.1.	Hazardous to the aquatic environment	None		None	Not assessed in this dossier
5.1.	Hazardous to the ozone layer	None		None	Not assessed in this dossier

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
[3)] Converted acute toxicity point estimate from Table 3.1.2 of CLP
[4)] LD_{50} value for the dermal route

Table 4: Proposed labelling based according to the CLP Regulation

	Labelling	**Wording**
Pictograms	GHS08 GHS06 GHS05	Health hazard Skull and crossbones Corrosion
Signal Word	Dgr	Danger
Hazard statements	H350 H301 H312 H318 H317 H336	May cause cancer Toxic if swallowed Harmful in contact with skin Causes serious eye damage May cause an allergic skin reaction May cause drowsiness or dizziness

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Butanone oxime was previously discussed by the Technical Committee for Classification and Labelling (TC C&L) according to Directive 67/548/EEC. The Working Group on the Classification and Labelling of Dangerous Substances ECB in Ispra agreed on 19-21 January 2000 that butanone oxime should be classified with Carc. Cat. 3; R40 - Xn; R21 - Xi; R41 - R43, and agreed that classification for toxicity to reproduction is not warranted. Further it was decided that symbol Xn; R-phrases 21-40-41-43; and S-phrases (2-) 13-23-26-36/37/39 shall be added, but no Nota or specific concentration limits shall be set. This agreement was considered final and the proposal was sent to the European Commission, and was enacted by the Technical Progress Committee (TPC) for possible inclusion in a future ATP (amendments on technical progress).

Butanone oxime is listed by Index number 616-014-00-0 in Annex VI, Part 3, and Table 3.1 (list of harmonised classification and labelling of hazardous substances) of CLP. To date butanone oxime is legally classified as: Acute Tox. 4*, H312: Harmful in contact with skin; Skin Sens. 1, H317: May cause an allergic skin reaction; Eye Dam. 1, H318: Causes serious eye damage; and Carc. 2, H351: Suspected of causing cancer.

In July 2012 butanone oxime was proposed for substance evaluation (SEV) in compliance with Article 44(1) of the REACH Regulation. Butanone oxime is a high production volume chemical (> 1000 t/a) and is widely used with high exposure for workers. The SEV led to the conclusion that the existing harmonised classification and labelling of butanone oxime in CLP Annex VI should be revised with regard to carcinogenicity. The existing information on the toxicity of butanone oxime indicates that butanone oxime also meets the criteria for classification and labelling for acute oral toxicity and for narcotic effects according to CLP. Moreover, the results from skin sensitisation testing are sufficient for the allocation of butanone oxime into a sub-category.

For the purposes of this dossier all registrations available in October 2016 have been taken into account.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal aims of an update of the existing harmonized classification and labelling of butanone oxime and of new entries with respect to human health hazards in CLP Annex VI.

Carcinogenicity

Butanone oxime is legally classified as carcinogen Category 2 according to CLP. The evaluation of the available data has verified the concern that a more severe classification regarding carcinogenicity is needed. The available data for carcinogenicity of butanone oxime do not comply with the legal classification of butanone oxime as carcinogen Category 2. In combined chronic toxicity/carcinogenicity studies in rats and mice exposed by inhalation to butanone oxime sufficient evidence of animal carcinogenicity was demonstrated. Two animal experiments using two species (rats and mice) resulted in clear evidence for carcinogenicity. A causal relationship has been established between butanone oxime and a statistically significant increased incidence of benign and malignant tumours. Being similar to OECD TG 453/EU B.33 both studies are well conducted and do not cast doubts about the relevance of the results. Tumour development was noted in rats and mice

exposed to relatively low concentrations of butanone oxime by inhalation for a period of up to two years.

Butanone oxime is thus considered to meet the criteria for classification and labelling as carcinogen Category 1B, H350 but not as Category 2, H351 according to CLP.

Acute toxicity - oral

The data from a preliminary dose range-finding study to a developmental toxicity study in rabbits have shown that butanone oxime induces lethality in this species (as assumed to haemolytic anaemia). In females (5/5) treated with 80 mg/kg bw butanone oxime starting on gestation day (GD) 6 mortalities were observed on GD8 until GD10. First deaths (2/5 females) occurred shortly after less than 48 hours after two dosages (cumulative 160 mg/kg bw). All 5 females were found dead until GD10. Taken together, from single dose studies in rats and from repeated dose studies in rabbits, rabbits appear to be more sensitive than rats to the acute toxic effects of butanone oxime. According to the Guidance on the application of the CLP-criteria, classification should be based on the lowest acute toxicity estimates (ATE) value available i.e. the lowest ATE in the most sensitive appropriate species tested. As specified in the guidance on haemolytic anaemia mortalities during days 0-3 in a repeated dose study should be considered for acute toxicity. Therefore, based on the LD_{50} value of ≤ 160 mg/kg bw and thus the ATE of 100 mg/kg bw (converted acute toxicity point estimate from table 3.1.2 of CLP) observed in a developmental toxicity study in rabbits, butanone oxime fulfils the criteria for classification as Acute Tox. 3, H301: Toxic if swallowed according to CLP (Annex I, Part 3, Table 3.1 Acute toxicity: 50 < Category 3 ≤ 300 mg/kg).

Acute toxicity - dermal

Based on the review of the available experimental data for acute dermal toxicity of butanone oxime, it is concluded that butanone oxime meets the criteria for classification and labelling as Acute Tox. 4, H312 (CLP) and the reference indicating minimum classification "(*)" is no longer necessary. The legal classification of butanone oxime is confirmed for Acute Tox. 4, H312 (Harmful in contact with skin).

Skin sensitisation

Butanone oxime has shown a clear evidence of skin sensitisation in guinea pigs (GPMT and Buehler assay). The results of a mouse ear swelling test (MEST) with butanone oxime have shown a sensitising response of 40 % and a swelling rate of 120 % for the mouse ear. Based on this animal model system a moderate potency for skin sensitisation is determined for butanone oxime. Based on available data, butanone oxime is classified as skin sensitizer category 1 (legal classification).

In comparison to the given criteria for the hazard category and sub-categories for skin sensitisation according to CLP butanone oxime fulfils the criteria for classification in the hazard class as skin sensitizer sub-category 1B, H317: May cause an allergic skin reaction, because a skin sensitisation response of ≥ 30 % at > 1.0 % i.d. induction dose was observed in the adjuvant type test method (GPMT); and of ≥ 15 % at > 20 % topical induction dose in the non-adjuvant type test method (Buehler assay).

Serious eye damage / eye irritation

The available data on serious eye damage/eye irritation do fulfil the criteria laid down in CLP, and the legal classification as 'Irreversible effects on the eye' Category 1, H318 is warranted.

Specific target organ toxicity – single exposure

Further the evaluation of data has verified the concern that butanone oxime caused transient target organ effects. There were changes in neurobehavioral function including narcotic effects. In acute oral, inhalation and dermal toxicity studies and also in studies with repeated exposure to butanone oxime in different animal species, transient and reversible changes in neurobehavioral function consistent with central nervous system depression, but no evidence of cumulative neurotoxicity was detected. Based on these data there is reasonable concern and butanone oxime should be classified additionally due to its narcotic effects according to CLP.

In rats single oral doses of ≥ 300 mg/kg bw butanone oxime administered by gavage produced narcotic effects. In the acute inhalation toxicity study with rats a strong transient narcotic effect occurred in both sexes at 4.83 mg/L/4h. In a dermal acute toxicity study in rabbits butanone oxime produced significant effects on the central nervous system (CNS) at single doses of 185 mg/kg bw and higher, and transient narcosis occurring during the first 48 hours following exposure at the low dose level of 18 mg/kg bw. Also in specific investigations transient and reversible functional disturbances in nervous system function consistent with CNS depression were observed in rats after single or repeated oral application of butanone oxime. Based on these data, butanone oxime meets the criteria for classification and labelling as a specific target organ toxicant (single exposure) of Category 3 for narcotic effects (STOT SE 3, H336: May cause drowsiness or dizziness) according to CLP (Annex I, Part 3.8.2.2.2).

In Table 5 the following hazard classes for butanone oxime are proposed.

Table 5: Proposed classification and labelling of butanone oxime according to CLP

Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictograms, Signal Word
Acute Tox. 3	H301: Toxic if swallowed	GHS06: Skull and crossbones, Danger
Acute Tox. 4	H312: Harmful in contact with skin	GHS07: Exclamation mark, Warning
Eye Dam. 1	H318: Causes serious eye damage	GHS05: Corrosion, Danger
Skin Sens. 1B	H317: May cause an allergic skin reaction	GHS07: Exclamation mark, Warning
Carc. 1B	H350: May cause cancer	GHS08: Health hazard, Danger
STOT SE 3	H336: May cause drowsiness or dizziness	GHS07: Exclamation mark, Warning

2.3 Current harmonised classification and labelling

Table 6: Entry in Annex VI of CLP

Classification		Labelling			Specific Concentration limits, M-Factors	Notes
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)		
Acute Tox. 4*	H312	H312		GHS07		
Skin Sens. 1	H317	H317		GHS05		
Eye Dam. 1	H318	H318		GHS08		
Carc. 2	H351	H351		Dgr		

2.4 Current self-classification and labelling

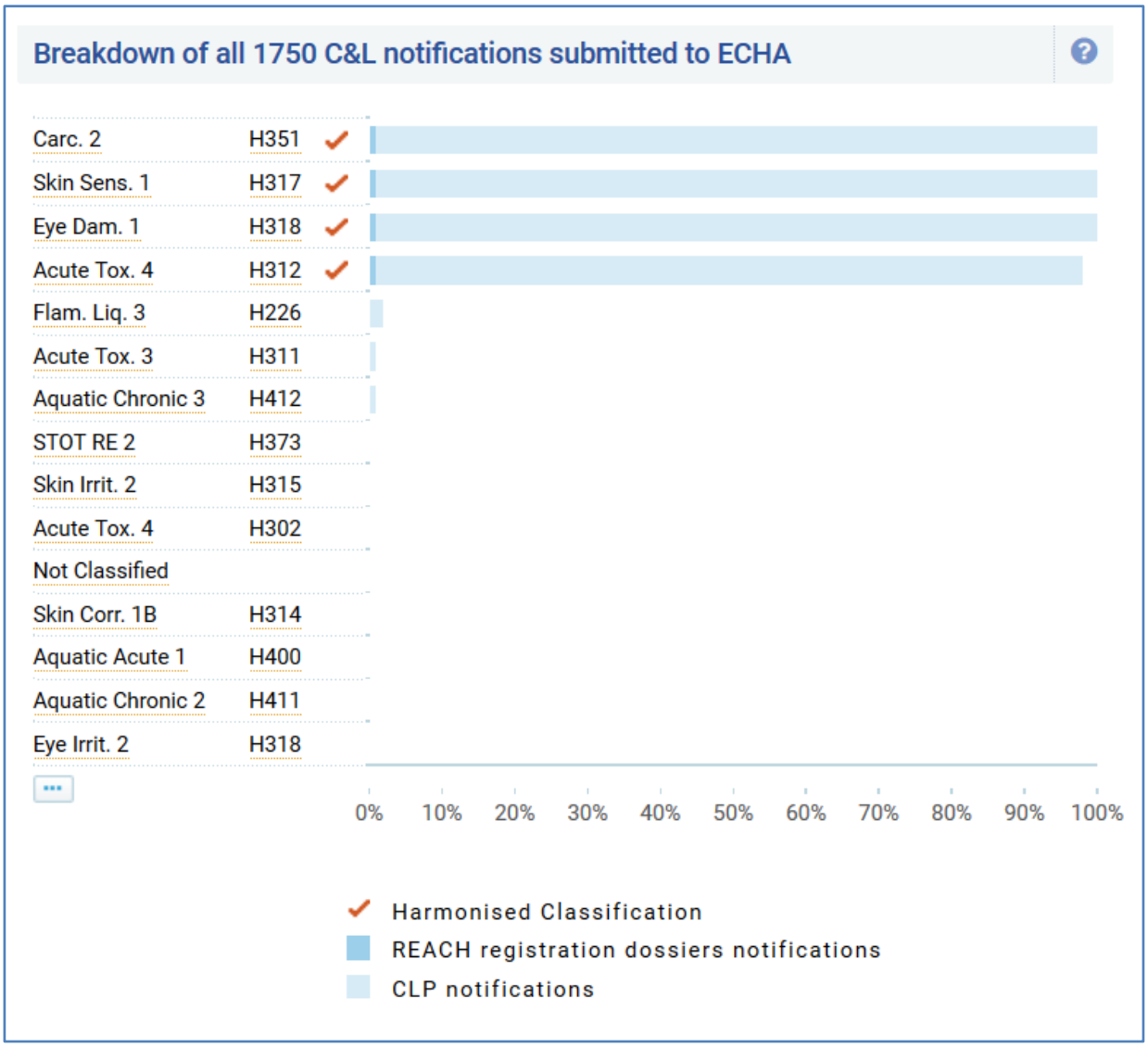

Figure 1: C&L notifications submitted to ECHA (www.echa.eu, May 2017)

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

In July 2012 butanone oxime was proposed for substance evaluation (SEV) in compliance with Article 44(1) of the REACH Regulation. The SEV led to the conclusion that the existing harmonised classification and labelling of butanone oxime in CLP Annex VI should be revised with regard to carcinogenicity. The existing information on the toxicity of butanone oxime further indicated that butanone oxime also meets the criteria for classification and labelling for acute oral toxicity and for narcotic effects according to CLP. Moreover, the results from skin sensitisation testing are sufficient for the allocation of butanone oxime into a sub-category.

Butanone oxime has CMR properties, thus a harmonized classification and labelling of this hazard class according to Article 36 of CLP is justified. Furthermore, a harmonized classification of the other proposed non-CMR hazard classes is also justified, since new hazard information was available suggesting that a change in harmonised classification is necessary. The self-classification of a substantial number of C&L notifiers is, moreover, diverging (16 different aggregated notifications in the C&L inventory), which also justifies the proposal for harmonised classification of the non-CMR hazard classes, especially regarding butanone oxime being a high production volume chemical (> 1000 t/a), which is widely used with high exposure for workers.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 7: Substance identity

EC number:	202-496-6
EC name:	butanone oxime
CAS number (EC inventory):	96-29-7
CAS number:	96-29-7
CAS name:	2-Butanone, oxime
IUPAC name:	butan-2-one oxime
CLP Annex VI Index number:	616-014-00-0
Molecular formula:	C_4H_9NO
Molecular weight range:	87.12 g/mol

Structural formula:

There is only one major isomer for butanone oxime (MEKO), which is trans/anti.

1.2 Composition of the substance

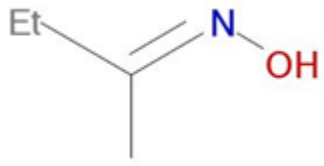

Table 8: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Butanone oxime EC-No.: 202-496-6	Please see confidential annex or technical dossier		

Current Annex VI entry:

Table 9: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
none			

Current Annex VI entry:

Table 10: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
none				

Current Annex VI entry:

1.2.1 Composition of test material

Please see 'Confidential Annex' or technical dossier

1.3 Physico-chemical properties

Table 11: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	*clear colourless liquid of organic origin*	*experimental result ASTM D 1209*	*CONDEA Servo BV 1995*
Melting/freezing point	*-29.5°C*	*OECD Guideline 102*	*Timmermans, 1921*
Boiling point	*> 152°C at 1013 kPa*	*OECD Guideline 103*	*Quitzsch et al. 1965*
Relative density	0.92 at 20°C		*CRC Handbook of Data on Organic Compounds 1985*
Vapour pressure	*1.07 kPa at 20°C* *0.14 kPa at 20°C*	*equivalent or similar to OECD Guideline 104*	*Wypych 2008* *NTP 1999*
Surface tension		*In accordance with Column 2 of REACH Annex VII, the study does not need to be conducted, as surface activity is not expected based on the structure of the substance; nor is it a desired substance property.*	
Water solubility	*100000 mg/L at 25°C and pH 7*	*OECD Guideline 105*	*Handbook of environmental data on organic chemicals 1983*
Partition coefficient n-octanol/water	*0.63 at 25°C*	*equivalent or similar to OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method)*	*EPIWIN Systpro Database 1992*
Flash point			
Flammability			
Explosive properties			
Self-ignition temperature			
Oxidising properties			

Granulometry	-	*In accordance with Column 2 of REACH Annex VII, the study does not need to be conducted, as the substance (a liquid) is manufactured and marketed in a non-solid form.*	
Stability in organic solvents and identity of relevant degradation products	*Butanone oxime is stable and miscible in alcohol, diethyl ether, CClF4, CCl2F2, CH2Cl2,*		*Copley et al. 1938*
Dissociation constant	*pKa: 12.45 at 25°C*	*equivalent or similar to OECD Guideline 112*	*King and Marion 1944*
Viscosity	*15 mPa s at 20°C*	*experimental result ASTM D 2196*	*OECD 2003*

The vapour pressure data show inconsistencies as a coefficient of 10 is between the given values. However, only few data are given in the technical dossier. For the value of 1.07 kPa a guideline is stated which is "equivalent or similar to OECD Guideline 104" However the exact method is not known. For the second value of 0.14 kPa no further information is stated in the technical dossier. However it is cited in studies of the US EPA and of the Canadian Environment. Therefore it could be assumed that this value should also be valid.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

By virtue of its anti-skinning properties butanone oxime is used in formulations of alkyd paints, primers, varnishes and coatings both for workers and consumers.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in the scope of this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

For basic toxicokinetics results from four experimental studies are available. For dermal absorption one study is available. In all studies butanone oxime was used as test material.

Basic toxicokinetics

In the studies of Burka et al. (1998) and NTP (1999) the extent of absorption was estimated. The disposition of ^{14}C-butanone oxime (purity: ~ 96 %) was determined in the male F344 rat following a single intravenous injection of 2.7 mg/kg bw butanone oxime. After administration, tissues and excreta of rats were collected and analysed for radioactivity. ^{14}C-butanone oxime was primarily excreted as CO_2 (48.8 %), via urine (21.4 %), and as exhaled volatiles (11.4 %). About 7 % of the administered radioactivity remained in the tissues after 72 hours. No tissue showed any marked accumulation of radioactivity.

The effect of dose on the rate and route of excretion of butanone oxime was also examined by Burka et al. (1998) and NTP (1999). The disposition of ^{14}C-butanone oxime was determined in the male F344 rat following single oral (gavage) administration of 2.7, 27, and 270 mg/kg bw. After administration, tissues and excreta of rats in each group were analysed for radioactivity. ^{14}C-butanone oxime was readily absorbed from the gastrointestinal tract and was extensively metabolized to CO_2 (50-70 %), mostly in the first 24 h after dosing. Excretion in urine increased with increasing dose and ranged from about 13 % (2.7 mg/kg bw) to 26 % (270 mg/kg bw). Respiratory excretion as volatiles was 5-18 % (increased as the dose increased). As excretion in CO_2 decreased with dose, excretion in urine and as volatiles increased. Excretion in faeces was less than 2 % for each dose. Total recoveries of radioactivity were approximately 90 % for each dose. Accumulation of radioactivity in the tissues was 5-7 % after 72 hours of dosing, with no tissue demonstrating any marked accumulation of radioactivity.

Metabolite profile in urine, 0-8 h after dosing of 270 mg/kg bw butanone oxime: 5 polar metabolites were identified that could only be partially resolved by anion exchange chromatography (CO_2, methyl ethyl ketone (MEK), glucurunides, and other polar metabolites). Incubation with glucuronidase, but not sulphatase, changed the urinary metabolic profile. MEK was a major component in the volatiles. The glucuronide conjugates of butanone oxime and its metabolites or other polar metabolites were primarily excreted in urine (Burka et al. 1998).

The biotransformation of butanone oxime (purity: 99.5 %) in comparison to acetoxime (CAS 127-06-0) in vivo and in vitro was evaluated and the capacity to catalyse these reactions was compared in different animal species and humans (Völkel et al. 1999; TL 22, 2000, unpublished study report, confidential). The biotransformation of butanone oxime was studied in liver microsomes and cytosol from male and female rats, mice and several human liver samples. The chemical reactivity of the postulated butanone oxime-metabolites was characterised. Butanone oxime was found to be oxidized to butane-2 nitronate by microsomal monooxygenases but at very low rates. No sex differences in the rates of microsomal oxidation of butanone oxime to butane 2-nitronate were noted. The hypothesized biosynthesis of methyl ethyl ketoxime *O*-sulfate or acetoxime *O*-sulfate in liver sub-cellular fractions from corresponding nitronates or oximes did not occur at all or occurred at very low rates since

formation of the stable *O*-sulfate could not be demonstrated using acetoxime and butanone oxime or propane 2-nitrate and butane 2-nitronate as substrates in the presence of appropriate cofactors.

Additionally, the ability of butanone oxime to induce DNA and RNA-modifications was studied in male and female rats exposed to butanone oxime by inhalation. No increase in modifications was detected in DNA isolated from rats exposed to butanone oxime of 1000 ppm (3.6 mg/L) for 6 hours. An increase in 8-aminoguanosine was observed in liver RNA from rats exposed to butanone oxime to a greater extent in males than in females.

The toxicokinetic studies demonstrated the existence of two and suggested a possible third metabolic pathway for butanone oxime in the rat, the major pathway being the hydrolysis of butanone oxime to MEK. One of the minor pathways appears to be a P450 mediated oxidation of butanone oxime to butane-2 nitronate and the second a reduction of butanone oxime. No quantitative sex differences in these pathways were identified.

The disposition of butanone oxime in Swiss Webster pregnant mice, which received a single oral dose of ^{14}C-butanone oxime on GD14 (Gestation day 14), was evaluated by autoradiography at selected time points: 20 minutes, 1, 3, 9 and 24 hours (TL 15, 1981, unpublished study report, confidential). Nasal epithelium and the liver had the highest concentrations of radioactivity. The nasal epithelium showed a remarkably rapid and persistent affinity for the material, high concentrations were found at all time intervals studied. Tissues which showed an increasing relative concentration with time were bone marrow, spleen, seromucous and salivary glands, Harder's gland, intestinal wall, mammary ducts, and foetus. The concentration in the pancreas peaked after 3 hours and then declined. There was apparent absorption of the compound and/or metabolites by the liver and kidney. Urine and bile contained considerable radioactivity throughout the course of the study. There was minimal radioactivity in the contents of the intestine.

Dermal absorption

The dermal absorption and disposition of ^{14}C-butanone oxime was determined in male F344 rats following single dermal administration of 2.7 and 270 mg/kg bw butanone oxime. Dose sites were protected from grooming by a non-occlusive foam appliance with a cloth cover and a metal shield. After administration, tissues, including application site and excreta of rats in each group were analysed for radioactivity (Burka et al. 1998; NTP 1999). In the 72 hours after dermal application of ^{14}C-butanone oxime, 13 % of the 2.7 mg/kg bw dose and 26 % of the 270 mg/kg bw dose were absorbed. No tissue demonstrated marked accumulations of radioactivity.

4.1.2 Human information

No information is available on absorption, distribution, metabolism, or excretion studies of butanone oxime in humans.

4.1.3 Summary and discussion on toxicokinetics

Data on the toxicokinetics of butanone oxime was obtained from animal testing. No data were found on the toxicokinetics of butanone oxime after exposure by inhalation. No information is available on the toxicokinetics of butanone oxime in humans.

Absorption

After a single oral (gavage) administration of 2.7, 27, or 270 mg/kg bw, ^{14}C-butanone oxime was readily absorbed (about 100 %) from the gastrointestinal tract and was primarily converted to CO_2 (71 %), mostly in the first 24 hours after dosing. During 72 hours of exposure, 13 % of a 2.7 mg/kg bw dose and 26 % of a 270 mg/kg dose were absorbed when administered dermally.

Distribution

Distribution of oral and intravenous administered dose of 2.7 mg/kg bw butanone oxime to rats were strikingly different, with less conversion to CO_2 in the intravenous application than by the oral administration, 49 % or 71 %, respectively. The decrease in excretion as CO_2 following intravenous administration was offset by increases in excretion in urine and as volatiles. A comparison of dermal and intravenous data indicates that the relative distribution of the absorbed doses in the dermal studies into urine, CO_2 and tissues was similar to those of the intravenous doses. After a single intravenous injection of 2.7 mg/kg bw butanone oxime to male F344 rats, about 7 % of the administered radioactivity remained in the tissues after 72 hours. The distribution of radioactivity into individual tissues was also similar to that found after intravenous administration. Radioactivity was detected in all examined tissues (adipose, blood, kidney, liver, muscle, skin and testis) 72 hours post-dosing. About 5-7 % of the administered radioactivity remained in the major tissues after 72 hours. None of the tissue demonstrated any marked accumulation of radioactivity. Therefore it can be concluded that butanone oxime does not accumulate in tissues.

In a further study the distribution of butanone oxime in Swiss Webster pregnant mice receiving a single oral dose of ^{14}C-butanone oxime on GD14 was evaluated. The highest concentrations of radioactivity were detected in the nasal epithelium and the liver. The nasal epithelium showed a rapid and persistent affinity for the material, and high concentrations were found at all-time intervals studied. Organs and tissues which showed an increasing relative concentration with time were bone marrow, spleen, seromucous and salivary glands, Harder's gland, intestinal wall, mammary ducts, and foetuses.

Metabolism

Butanone oxime is extensively metabolized, yielding CO_2, MEK, glucuronides, and other polar metabolites, and does not accumulate in tissues. The toxicokinetic studies of butanone oxime demonstrated the existence of two metabolic pathways and the possibility of a third one (based on acute oral, dermal and intravenous doses only). The major pathway is the hydrolysis of butanone oxime to MEK, and the second pathway is the oxidation of butanone oxime to butane 2-nitronate by microsomal monooxygenases, but this occurs at very low rates. No sex differences in the capacity to oxidize butanone oxime were observed in the species examined.

Excretion

Single oral doses of butanone oxime given at 2.7, 27 and 270 mg/kg bw to rats were extensively converted to CO_2 (~ 50-70 %), mostly in the first 24 hours post-dosing. Excretion in urine increased with increasing dose, and ranged from 13 % of the low dose to 26 % of the high dose. Excretion as volatiles was only 5-7 % of the dose for the two lower doses, but comprised an average of 18 % for the high dose. CO_2 remained the major metabolite as the dose increased, less of the administered dose was excreted as CO_2 and relatively more was excreted in urine and as volatiles. Excretion in the faeces was < 2 % for each dose level.

Overall, ^{14}C-butanone oxime was readily absorbed from the gastrointestinal tract and the skin, underwent widespread uptake, was distributed over the entire body, was extensively metabolized to CO_2 and MEK, which were excreted via the lungs, and glucuronide conjugates of butanone oxime and its metabolites or other polar metabolites, which were excreted in urine and bile, and was not accumulate in tissues. Distribution of butanone oxime was clearly different following intravenous application compared with oral administration, particularly in the first few hours. Nearly twice as much radioactivity was eliminated by the first time point following oral administration of 2.7 mg/kg bw compared with intravenous application at the same dose, suggesting that a substantial portion of butanone oxime undergoes first-pass metabolism following oral administration. Following dermal administration, significantly greater amounts of volatiles were excreted than after gavage or intravenous administration. Butanone oxime and its metabolites were primary excreted via urine.

Butanone oxime biotransformation appears to be complex. The first activating step (likely P450 dependent oxidation) may have only a minor and dose-dependent contribution to the overall biotransformation of butanone oxime. The results indicate that nitronate formation alone is not sufficient to explain the carcinogenicity of butanone oxime.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

For the acute oral toxicity of butanone oxime results from 4 experimental studies in rats are used for evaluation. Additionally, mortalities observed in repeated dose toxicity studies during days 0-3 were considered relevant for assessing the acute toxicity of a compound. For that reason, the data from a developmental toxicity study (range-finding and main study) in rabbits (oral, gavage) (TL19, 1990b, unpublished study report, confidential; Derelanko et al. 2003) are discussed as deaths in animals were observed in these studies within 48 hours after first administration. In all studies butanone oxime was used as test material.

The results of all available experimental studies on acute toxicity after oral administration of butanone oxime are summarised in Table 12.

Table 12: Butanone oxime: Studies on acute oral toxicity (gavage)

Reference **Species; strain; sex; method**	**LD_{50} / Results**
TL8 (1991), unpublished study report, confidential; Schulze and Derelanko (1993) **Rat**; Sprague-Dawley; male, female (10/sex/dose) acute neurotoxicity study, GLP compliant Test substance: butanone oxime purity: confidential (> 98 %)	**$LD_{50, rat\ m/f}$ > 900 mg/kg bw** Doses tested: 0, 100, 300 and 900 mg/kg bw 900 mg/kg bw (highest dose): no mortality; decreased activity 30-60 min after exposure LOEL = 300 mg/kg bw: based on transient neurobehavioral effects (impaired gait, disturbed aerial righting reflex, reversible within 24h); suggested a transient narcoleptic response

TL1 (1978a), unpublished study report, confidential **Rat**; Sprague-Dawley; male, (> 9/dose) protocol similar to OECD TG 401/EU B.1, GLP compliant Test substance: butanone oxime purity: 99.5 %	**$LD_{50, male\ rat}$ ca. 2326 mg/kg bw** Doses tested: 0, 1500, 1908, 2427, 3089 and 4999 mg/kg bw 1500 mg/kg bw: no mortality; 1908 mg/kg bw: 22.2 % deaths 2427 mg/kg bw: 50 % deaths ≥ 3089 mg/kg bw: 100 % deaths. Mortality occurred within 48 hours.
TL3 (1971a), unpublished study report, confidential **Rat**; strain, sex and no. of animals not specified; in-house protocol, no GLP compliance Test substance: butanone oxime purity: confidential (> 98 %)	**$LD_{50, rat}$ ca. 2528 mg/kg bw** Doses tested and specific results not reported
TL5 (1982), unpublished study report, confidential **Rat**; Sherman-Wistar; male, female (5/sex/dose) method not specified, GLP compliant Test substance: butanone oxime purity: confidential (> 98 %)	**$LD_{50, male\ rat}$ ca. 930 mg/kg bw** (95 % confidence: 670 - 1310 mg/kg bw) **$LD_{50, female\ rat}$ ca. 1620 mg/kg bw** (95 % confidence: 1230 - 2140 mg/kg) Doses tested: 0, 250, 500, 1000, 2000 and 4000 mg/kg bw Most deaths occurred within a few hours. A very steep dose-response was seen in males as well as in females (mortality rate: m: 0 % at 500 mg/kg, and 80 % at 1000 mg/kg bw f: 0 % at 1000 mg/kg bw, and 80 % at 2000 mg/kg bw).
TL19 (1990b), unpublished study report, confidential; Derelanko et al. (2003) **Key study** **Rabbit;** New Zealand White; female; (5/group in preliminary study; 18/group in main study) According to OECD TG 414 / EU B.31 (Developmental toxicity study), GLP compliant Test substance: butanone oxime	*Preliminary study (dose range-finding study):*

purity: confidential (> 98 %) Exposure duration: GDs 6-18 (daily)	80 mg/kg bw/d for 2 days **(cumulative 160 mg/kg bw)** induced **mortality:** ≤ 48h in 2/5 females deaths between GD8-10 all 5/5 females Clinical signs: dark red or reddish-green coloured urine, enlarged spleen, brown discoloured lungs **$LD_{50,\ female\ rabbit}$ ≤ 160 mg/kg bw** 40 mg/kg bw/d for 4 days **(cumulative 160 mg/kg bw)**: induced mortality in 2/5 females (GD10-11) **$LD_{50,\ female\ rabbit}$ ≤ 160 mg/kg bw** According to the CLP Regulation (Annex I, Part 3, Table 3.1.2 Acute oral toxicity: 50 < Category 3 ≤ 300 mg/kg bw, converted acute toxicity point estimate/ATE = 100 mg/ kg bw) butanone oxime fulfils the criteria for classification as **Acute Tox. 3, H301: Toxic if swallowed** *Main study:* 40 mg/kg bw/d for 5 days **(cumulative 200 mg/kg bw)**: induced **mortality** in 8/18 females **(**GD11-24); clinical signs (decreased activity, wobbly gait, no faeces, greenish or reddish coloured fluid in the cage/tray, urine stains, emaciation, pale eyes and ears, and yellowish coloured crusty material in the nostrils); ↓: bw and food consumption; necropsy: fluid contents in the thoracic cavity, brown discoloration of the lungs, mucoid material attached to the mucosa of the stomach, pale liver, accentuated lobular markings on the liver, urinary bladder with dark red fluid contents and thickened mucosa **$LD_{50,\ female\ rabbit}$ ≤ 200 mg/kg bw** (estimate, not calculated)

4.2.1.2 Acute toxicity: inhalation

For the acute inhalation toxicity of butanone oxime results from two experimental studies in rats are used for evaluation. In both studies butanone oxime was used as test material. The results of experimental studies on acute inhalation toxicity to butanone oxime are summarised in Table 13.

Table 13: Butanone oxime: Studies on acute inhalation toxicity (vapour, whole body)

Reference **Species; strain; sex; method**	**LC_{50} / Results**
TL2 (1984a), unpublished study report, confidential **Rat**; F344; male, female (5/sex/group) protocol similar to OECD TG 403/EU B.2, GLP compliant Test substance: butanone oxime purity: confidential (> 98 %) Exposure duration: 4 h	**$LC_{50,\ rat}$ > 4.83 mg/L/4h** (analytical) (male/female); **no mortality** Concentrations tested: 0, 0.19, 1.45 and 4.83 mg/L LOAEC = 190 mg/m^3 based on statistically significant decreased bw gain in females in the observation period after exposure (14 days) LOAEC = 1450 mg/m^3 based on methaemoglobin formation LOAEC = 4800 mg/m^3 based on evidence of narcotic effects

TL3 (1971b), unpublished study report, confidential **Rat**; strain not specified; male, female (6/sex/group); in-house protocol, no GLP compliance Test substance: butanone oxime purity: confidential (> 98 %) Exposure duration: ca. 8 h	**$LC_{50, rat}$ > 13.2 mg/L/4h** (calculated, modified Haber's law: Cn * t = const, where C = concentration, t = exposure duration, and n = 3); no mortality at 10.5 mg/L (highest tested concentration; male/female)

4.2.1.3 Acute toxicity: dermal

For the acute dermal toxicity of butanone oxime results from two experimental studies in rabbits were used for evaluation. In both studies butanone oxime was used as test material. The results of experimental studies on acute toxicity after dermal exposure of butanone oxime are summarised in Table 14.

Table 14: Butanone oxime: Studies on acute dermal toxicity (occlusive coverage)

Reference **Species; strain; sex; method**	**LD_{50} / Results**
TL19 (1991), unpublished study report, confidential **Rabbit;** New Zealand White; male, female (5/sex/dose); protocol similar to OECD TG 402/EU B.3, GLP compliant Test substance: butanone oxime purity: confidential (> 98 %) Exposure duration: 24 h	**$LD_{50, rabbit, m/f}$ > 1000 mg/kg bw** (Limit Test) Doses tested: 1000 mg/kg bw no mortality within a 14 day period after dosing
TL2 (1984b), unpublished study report, confidential **Key study** **Rabbit**; New Zealand White; male, female (5/sex/dose); protocol similar to EPA OTS 798.1100 Guideline, GLP compliant Test substance: butanone oxime Purity: 99.5 % Exposure duration: 24 h	**$LD_{50, rabbit, m/f}$ = 1848 mg/kg bw** Doses tested: 0.02, 0.2 and 2.0 mL/kg bw (18, 185 and 1848 mg/kg bw) 2.0 mL/kg equivalent to 1848 mg/kg bw (calculation based on density of 0.924 g/mL); **mortality within 48h** LOAEL = 185 mg/kg bw, based on methaemoglobin formation, and splenic erythrophagocytosis LOEL = 18 mg/kg bw based on reversible narcotic effects According to the CLP Regulation (Annex I, Part 3, Table 3.1.1 and 3.1.2 Acute toxicity: 1000 < Category 4 ≤ 2000 mg/kg bw, LD50/ATE = 1848 mg/kg bw) butanone oxime fulfils the criteria for classification as **Acute Tox. 4, H312: Harmful in contact with skin**

4.2.1.4 Acute toxicity: other routes

No data were submitted.

4.2.2 Human information

No information is available on the acute toxicity of butanone oxime in humans.

4.2.3 Summary and discussion of acute toxicity

Data for acute toxicity of butanone oxime was obtained from animal testing. To evaluate the hazard class acute oral toxicity of butanone oxime, e.g. derivation of the LD_{50} value, data from a developmental study in rabbits were also considered. No information is available on the acute toxicity of butanone oxime in humans.

Acute oral toxicity studies with butanone oxime in rats resulted in the following LD_{50} values: > 900 mg/kg bw (male/female); approx. 2326 mg/kg bw (male); and approx. 2528 mg/kg bw (male/female). Lower values were reported from a fourth study: approx. 930 mg/kg bw in males and approx. 1620 mg/kg bw in females.

Taken together, comparing these LD_{50}-values from the rat with rabbit data, the rabbit appears to be more sensitive than the rat to the toxic effects of butanone oxime. Data from a developmental toxicity study in rabbits were included in the evaluation of the acute oral toxicity of butanone oxime indicating that acute mortalities were observed in female rabbits treated with butanone oxime during the gestation phase. In a preliminary dose range-finding study all 5 rabbits receiving doses of 80 mg/kg bw starting on GD6 were found dead between the GD8 to 10. First deaths (2/5 females) occurred extremely short after less than 48 hours after two dosages (cumulative 160 mg/kg bw). At necropsy animals showed dark red or reddish-green coloured urine, enlarged spleen, and brown discoloured lungs. The treatment with 40 mg/kg bw during the GD6 to 9 (four days) was fatal for 2/5 females. In the main study, the treatment with 40 mg/kg bw during the GD6 to 10, five days induced mortality in females starting on the GD11. A total of 8 from 18 females were found dead on GD24. Animals showed the following clinical signs: decreased activity, wobbly gait, no faeces, greenish or reddish coloured fluid in the cage/tray, urine stains, emaciation, pale eyes and ears, and yellowish coloured crusty material in the nostrils); decreases in body weight and food consumption; necropsy: fluid contents in the thoracic cavity, brown discoloration of the lungs, mucous material attached to the mucosa of the stomach, pale liver, accentuated lobular markings on the liver, urinary bladder with dark red fluid contents and thickened mucosa. Based on the available data it is concluded that butanone oxime is acutely toxic after oral application. The LD_{50} value for butanone oxime was not calculated but roughly estimated as being ≤ 160 mg/kg bw (2 x 80 mg/kg bw) based on 2/5 deaths until 48 hours after dosing and on lethality of all 5 females until 72 hours after dosing.

Acute inhalation toxicity: In the available studies the exact LC_{50} value for butanone oxime could not be established as no death in male and female rats occurred in these studies. In one study no lethality or signs of evident toxicity were noted at the highest vapour concentration tested of 4.83 mg/L following 4 hour whole body exposure. In another inhalation hazard test no mortality was observed after an 8 hour whole body exposure to the highest tested concentration of 10.5 mg/L butanone oxime as a vapour. The LC_{50} value was found to be > 10.5 mg/L/8h. This value was extrapolated to a 4 hour exposure by using the modified Haber's law (Cn * t = const, where C = concentration, t = exposure

duration, and n = 3) for extrapolation from longer to shorter exposure durations. For a 4 hour exposure a LC_{50} value for butanone oxime of higher than 13.2 mg/L/4h was calculated. Because no mortalities were noted up to 13.2 mg/L/4h it is concluded that butanone oxime is not acutely toxic by inhalation.

Acute dermal toxicity: The dermal LD_{50} for rabbits was between 1000 and 1848 mg/kg bw butanone oxime. In one study no mortality was observed at 1000 mg/kg bw (Limit Test). After topically administration of a single dose of 185 mg/kg bw by an occlusive dressing for 24 hours methaemoglobin formation and splenic erythrophagocytosis was observed. At 2.0 mL/kg bw, equivalent to 1848 mg/kg bw (calculation based on density of 0.924 g/mL) all animals died within 48 hours after treatment. Based on the available data it is concluded that butanone oxime is acutely toxic after dermal administration.

4.2.4 Comparison with criteria

Acute toxicity means those adverse effects occurring following oral or dermal administration of a single dose of a substance or a mixture, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours. Acute toxicity relates to effects occurring after a single or relative brief exposure to a substance or mixture. Acute toxicity classification is generally assigned on the basis of evident lethality. The evidence for acute toxicity of butanone oxime is obtained from animal testing. Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the criteria shown in the Table 3.1.1 and 3.1.2 of Annex I, Part 3.

Since the Commission has recently proposed a notation for ATE values in Table 3.1 of CLP (10^{th} ATP to CLP), introducing harmonised ATEs in the same column as the SCLs and M-factors, specific ATEs and/or converted acute toxicity point estimate values according to Table 3.1.2 of CLP have to be used for classification purposes.

Acute oral toxicity: The following applies for the classification as

'*Acute oral toxicity -50 < Category 3 ≤ 300 mg/kg bw'*

'Acute oral toxicity -300 < Category 4 ≤ 2000 mg/kg bw.'

Based on the lowest oral LD_{50} values (about 930 mg/kg bw for males, and 1620 mg/kg bw for females) from studies with rats, butanone oxime would fulfil the criteria for classification for acute oral toxicity Category 4. However, the data from a preliminary dose range-finding study to a developmental toxicity study in rabbits (TL19, 1990b, unpublished study report, confidential; Derelanko et al. 2003) have shown that butanone oxime induces acute lethality in this species at lower doses. In female rabbits treated with 80 mg/kg bw butanone oxime starting on GD6 mortalities were observed between the GD8-10. First deaths (2/5 females) occurred extremely shortly after less than 48 hours after two dosages (on GD6 and 7, cumulative 160 mg/kg bw), and the remaining 3/5 females were found dead up to GD10. These mortalities occurring within the first 48 hours after dosing are taken as relevant for the classification proposal on acute oral toxicity.

In the synopsis of the available data from single dose studies in rats and from repeated dose studies in rabbits, the rabbit appears to be more sensitive than the rat to the acute toxic effects of butanone oxime. According to the Guidance on the application of the CLP-criteria, classification should be based on the lowest ATE/LD_{50} value available i.e. the lowest ATE/LD_{50} in the most sensitive

appropriate species tested. As specified in the guidance on haemolytic anaemia mortalities during days 0-3 in a repeated dose study should be considered for acute toxicity. Based on these data it is concluded that butanone oxime is acutely toxic by oral application (CLP Guidance, 3.9.2.5.2. Haematotoxicity).

For the oral route, the estimated ATE/LD_{50} is ≤ 160 mg/kg bw. Since there is no exact experimentally-derived LD_{50} value, the appropriate conversion from Table 3.1.2 of CLP (Annex I, Part 3) to a converted acute toxicity point estimate that relates to a classification category is used. According to Table 3.1.2 of CLP (50 < Category 3 ≤ 300 mg/kg bw), the converted acute toxicity point estimate and thus ATE for oral toxicity is 100 mg/kg bw. Based on this derived ATE, butanone oxime fulfils the criteria for classification as acute hazard Category 3 (Acute Tox. 3, H301: Toxic if swallowed).

Acute inhalation toxicity: The following applies in comparison to

'Acute inhalation toxicity (vapour): 10 < Category 4 ≤ 20.0 mg/L.'

No LC_{50} value could be established for butanone oxime as **no deaths** occurred in an inhalation hazard test up to a concentration of 10.5 mg/L/8h (corresponding to 13.2 mg/L/4h by using the modified Haber's law) butanone oxime as a vapour (TL3, 1971b, unpublished study report, confidential). At this highest tested concentration in the study no compound related signs of overt toxicity rather than lethality as indications of acute inhalation toxicity were observed. There are no data available that could justify a classification on acute inhalation toxicity according to CLP.

Acute dermal toxicity: The following applies for the classification as

'Acute dermal toxicity -1000 < Category 4 ≤ 2000 mg/kg bw.'

The current Annex VI entry for butanone oxime includes a classification for acute toxicity Category 4 with hazard statement H312 (Harmful in contact with skin) as a minimum classification as indicated by the reference '*' in the column "Classification" in Table 3.1. Based on the review of the available experimental data for acute dermal toxicity for butanone oxime, it is confirmed that butanone oxime meets the criteria for classification and labelling as Acute Tox. 4, H312 according to CLP and the reference indicating minimum classification (*) is no longer necessary. Reliable LD_{50} values for classification of butanone oxime were derived from acute dermal toxicity studies in rabbits. In the key study (TL19, 1991, unpublished study report, confidential), performed to a comparable protocol as OECD TG 402/EU B.3, no lethality was observed at the highest dose tested of 1000 mg/kg bw. In the second study (TL2, 1984b, unpublished study report, confidential), the dermal LD_{50} was found to be 1848 mg/kg bw. Based on this ATE/LD_{50} value, butanone oxime meets the criteria for classification as Acute Tox. 4, H312 according to CLP (Annex I, Part 3, Table 3.1.1 and 3.1.2 Acute toxicity (dermal): 1000 < Category 4 ≤ 2000 mg/kg bw).

4.2.5 Conclusions on classification and labelling

According to CLP butanone oxime has to be classified as:

Acute Tox. 3 for oral exposure and labelled with hazard statement H301: Toxic if swallowed.; with the pictogram "GHS06: Skull and crossbones", and with the signal word "Danger"; and

Acute Tox. 4 for dermal exposure and labelled with hazard statement H312: Harmful in contact with skin; with the pictogram "GHS07: Exclamation mark", and with the signal word "Warning".

Butanone oxime has not to be classified as acutely toxic by inhalation according to CLP.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute **oral** toxicity data for butanone oxime are available from 4 experiments carried out in rats. In addition, mortalities were observed in a repeated dose developmental toxicity study in rabbits, occurring within 48 h of dosing.

The lowest LD_{50} in the acute oral toxicity studies was 930 mg/kg bw (male rats), however a higher degree of lethality was seen in the rabbit developmental toxicity study. In a preliminary dose-range finding study, 5/5 pregnant rabbits died within 4 days of receiving two daily doses of 80 mg/kg bw/day butanone oxime. Two of these females died less than 48 h after receiving the second dose (cumulative dose = 160 mg/kg bw/day). At the next lowest dose of 40 mg/kg bw/day mortality was also observed, in both the preliminary and the main study. The LD_{50} for butanone oxime is estimated to be ≤ 160 mg/kg bw.

Based on the results of the developmental toxicity study in rabbits, the estimated oral ATE value for butanone oxime is ≤ 160 mg/kg bw. Using Table 3.1.2 of CLP, the appropriate classification is category 3 (50 < ATE ≤ 300 mg/kg bw) and the converted acute toxicity point estimate (cATpE) is 100 mg/kg bw. Therefore, butanone oxime should be classified as Acute Tox. 3; H301 (Toxic if swallowed).

There are 2 rat acute **inhalation** studies available. In the first, butanone oxime was tested up to a maximum vapour concentration of 4.83 mg/L for 4 h using whole body exposure. In the second study, rats were exposed to a maximum vapour concentration of 10.5 mg/L for 8 h (whole body) (extrapolated to a 4 h exposure to give an LC_{50} > 13.2 mg/L/4h). There were no deaths in either of these studies and therefore no classification is required.

There are two acute **dermal** toxicity studies in rabbits available. In the first study, no mortality was observed at a limit dose of 1 000 mg/kg bw. In the second, doses of 18, 185 and 1 848 mg/kg bw were applied to the skin of New Zealand White rabbits. At the top dose of 1 848 mg/kg bw all 5/5 animals died within 48 h of application. There were no deaths at the mid dose of 185 mg/kg bw. The LD_{50} therefore lies between 1 000 and 1 848 mg/kg. This meets the criteria for the classification Acute Tox. 4, H312 (category 4: 1 000 < ATE ≤ 2 000 mg/kg bw).*

Therefore, the dossier submitter (DS) has proposed that butanone oxime should be classified as Acute Tox. 3, H301: Toxic if swallowed and Acute Tox. 4, H312: Harmful in contact with skin. Butanone oxime was not acutely toxic following exposure by inhalation.

**The DS cited the LD_{50} in the second study to be 1 848 mg/kg bw. However this was clearly in error because at 1 848 mg/kg bw there was 100 % mortality.*

Comments received during public consultation

Six comments were received directly addressing acute oral toxicity. One Member State (MS) agreed with the proposed classification but there were five comments querying it. Of these five, three comments came from Industry questioning the lack of use of human experience and argued for a consideration of the low risk following use of butanone oxime as an anti-skinning agent in paints.

Two MS suggested that a study of developmental study in pregnant rabbits was inappropriate for assessment of acute oral toxicity. The lower LD_{50} obtained from this study may have been due to a higher sensitivity of pregnant animals rather than rabbits being the more sensitive species. These MS believed that the lowest LD_{50} of 930 mg/kg bw obtained in the rat studies was more appropriate for classification purposes, and that butanone oxime should be classified as Acute Tox. 4, H302.

All three MS agreed with the proposed classification for acute dermal toxicity.

Three comments (2 MS and 1 industry) were received specifically in support of no classification for inhalation toxicity.

Assessment and comparison with the classification criteria

Oral toxicity

The CLH report describe four acute toxicity studies in rats and a developmental toxicity study in rabbits.

In a study conducted in 1971 using an in-house protocol and an unspecified strain and number of rats (males and females according to the REACH registration document), the combined LD_{50} was 2 528 mg/kg bw.

In a 1978 study, carried out following a protocol similar to OECD guidelines and according to GLP, Sprague-Dawley rats (> 9 males/dose) were given a single oral dose of 0, 1 500, 1 908, 2 427, 3 089 or 4 999 mg/kg bw butanone oxime. There were no deaths at 1 500 mg/kg bw, 50 % mortality at 2 427 mg/kg bw and all animals died within 48 h following doses of ≥ 3 089 mg/kg bw. An LD_{50} value of 2 326 mg/kg bw was calculated by the study authors.

In a 1982 study, carried out to an unspecified method and GLP, male and female Wistar rats (5/sex/dose) were given a single oral dose of 0, 250, 500, 1 000, 2 000 or 4 000 mg/kg bw. The dose-response relationship was very steep. In males, 0 deaths occurred at 500 mg/kg bw, but 80 % mortality was observed at 1 000 mg/kg bw. In females, 0 deaths occurred at 1 000 mg/kg bw and 80 % mortality was seen at 2 000 mg/kg bw. LD_{50} values of 930 and 1 620 mg/kg bw were derived for males and females, respectively.

In an acute neurotoxicity study conducted in 1993 according to test guidelines and GLP. Sprague-Dawley rats (10/sex/dose) were given a single oral dose of 0, 100, 300 or

900 mg/kg bw. At the highest dose tested, there was no mortality and therefore the LD_{50} was > 900 mg/kg bw in this study.

In addition, acute toxicity of butanone oxime was evident in a rabbit developmental study. New Zealand White rabbits were administered 0, 10, 20, 40 or 80 mg/kg bw/day in the preliminary study (5 females/dose) and 0, 8, 14, 24 or 40 mg/kg bw/day in the main study (18 females/dose). The exposure duration was gestational days (GD) 6-18 (12 days).

Mortality was observed in both the preliminary study and the main study. In the preliminary study, 2/5 females died within 48 h of receiving 80 mg/kg bw/day and by GD 8-10 all five females had died. Clinical signs in this group included dark red or reddish/green coloured urine and necropsy revealed enlarged spleens and brown, discoloured lungs. Mortality was also observed at the 40 mg/kg bw/day in both the preliminary study and the main study. In the preliminary study 2/5 females died between GD 10-11 and in the main study 8/18 females died between GD 11-24. At 40 mg/kg bw/day clinical signs included decreased activity, laboured breathing, reddish coloured fluid in the bottom of the cage and decreased body weight. Brown discolouration of the lungs was noted, as were fluid contents in the thoracic cavity, pale liver, accentuated lobular markings on the liver, dark red contents in the urinary bladder and thickened mucosa.

According to OECD test guidelines, animals used for acute toxicity testing should be nulliparous and non-pregnant. However, the guidance to the CLP does not indicate that studies involving pregnant animals cannot be used. Data from any species can be used to contribute to acute toxicity classification and in general, classification should be based on the lowest ATE value available.

An LD_{50} was not determined. The greatest toxicity was seen at 80 mg/kg in the preliminary study, at which 2 deaths occurred within 48 h, following a cumulative dose of 160 mg/kg bw (2 × 80 mg/kg bw) and the remaining 3 dams died within 4 days following a maximum cumulative dose of 320 mg/kg bw. This would suggest an LD_{50} > 160, but < 320 mg/kg bw, and was estimated by RAC to be closer to 240 mg/kg bw. This would give rise to classification within Category 3 (50 < ATE ≤ 300 mg/kg). Using Table 3.1.2 of Annex I to the CLP Regulation, the converted acute toxicity point estimate (cATpE) would be 100 mg/kg bw.

Therefore, RAC agrees with the Dosser Submitter that in this case, classification for acute oral toxicity should be based on the lowest ATE value obtained, which is 100 mg/kg bw in pregnant rabbits. This corresponds to category 3: 50 < ATE ≤ 300 mg/kg bw.

Inhalation toxicity

Two acute inhalation toxicity studies in rats are available.

In a 1971 study, following an in-house protocol (non-GLP), male and females rats (strain unspecified) were exposed to a saturated atmosphere of butanone oxime vapour (10.5 mg/L) for 8 hours. There were no mortalities in this study. The LC_{50} was therefore > 10.5 mg/L (8-hour exposure). Extrapolation to a 4 hour exposure equivalent using Haber's law ($C^n \times t = k$) yields an LC_{50} of > 13.2 mg/L (4 hour exposure).

In a study dated 1984, following a protocol similar to OECD guidelines and GLP, male and females F344 rats were exposed to vapour concentrations of 0, 0.19, 1.45 and 4.83 mg/L for 4 hours (whole body). There was no mortality observed up to the top concentration of 4.83 mg/L, therefore the LC_{50} in this study was > 4.83 mg/L.

As there were no mortalities following inhalation exposure to butanone oxime in either of the studies available, the ATE derived is > 13.2 mg/L – **no classification for acute inhalation toxicity is necessary**.

Dermal toxicity

There are two acute dermal toxicity studies in rabbits available.

In a 1984 study, carried out to a protocol similar to guidelines and according to GLP, New Zealand White rabbits (5/sex/dose) received a topical dose of 18, 185 or 1 848 mg/kg bw occlusively to non-abraded skin for 24 hours. At the top dose of 1 848 mg/kg bw all 5/5 males and 5/5 females died within 48 hours of application. No rabbits died at any other doses. The LD_{50} in this study is therefore > 185 mg/kg bw, but < 1 848 mg/kg bw.

In the second study, carried out in 1991 according to a guideline similar to OECD and according to GLP, New Zealand White rabbits (5/sex/dose) were given a limit dose of 1 000 mg/kg bw, applied to occlusively to the dorsal area of trunk. Animals were exposed for 24 hours and a 14 day observation period followed. No deaths occurred during the course of this study.

The results of the two dermal studies in rabbits indicated that, similarly to the 1982 oral study in rats, the dose-response following exposure to butanone oxime via the dermal route is very steep. No deaths were seen up to doses of 1 000 mg/kg bw but at 1 848 mg/kg bw 100 % mortality was observed. The dermal LD_{50} lies between 1 000 and 1 848 mg/kg bw. As an exact ATE cannot be determined, a converted acute toxicity point estimate (cATpE) must be used. The LD_{50} determined falls in category 4 for acute dermal toxicity: 1 000 < ATE ≤ 2 000 mg/kg bw. According to the CLP Regulation, this corresponds to a cATpE of 1 100 mg/kg bw.

Conclusions on classification and labelling

In line with the DS's proposal, RAC considers that butanone oxime should be classified as

- **Acute Tox. 3; H301 (Toxic if swallowed)** based on a **cATpE of 100 mg/kg bw** derived from rabbits.
- **No classification for inhalation** is proposed and
- **Acute Tox. 4; H312 (Harmful in contact with skin)** is appropriate for . However, the dermal toxicity classification should be **based on a cATpE of 1 100 mg/kg bw**, and not 1 848 mg/kg bw as proposed in the CLH report.

4.3 Specific target organ toxicity – single exposure (STOT SE)

In acute oral, inhalation and dermal toxicity studies on butanone oxime, transient and reversible changes in neurobehavioral function consistent with central nervous system depression, but no evidence of cumulative neurotoxicity was detected. In repeated dose toxicity studies similar observations were noted after application of the test substance in different animal species. The results of experimental studies regarding narcotic effects of butanone oxime are summarised below.

Table 15: Butanone oxime: Narcotic effects observed in experimental animals

Reference **Species; strain; sex; method**	**Study results / Narcotic effects**
TL8 (1991), unpublished study report, confidential; Schulze and Derelanko (1993) **Rat**; Sprague-Dawley; male/female (10/sex/dose); **acute oral neurotoxicity study** (gavage), GLP compliant Test substance: butanone oxime purity: confidential (> 98 %) Doses tested: 0, 100, 300, 900 mg/kg bw	transient neurobehavioral effects (impaired gait; disturbed aerial righting reflex; reversible within 24h); suggested a transient narcoleptic response **LOEL $_{rat\ m/f}$ = 300 mg/kg bw** based on transient narcotic effects
TL2 (1984a), unpublished study report, confidential **Rat**; F344; male/female (5/sex/group); **acute inhalation toxicity study**, vapour, 4 h**;** protocol similar to OECD TG 403/EU B.2, GLP compliant Test substance: butanone oxime purity: confidential (> 98 %) Concentrations tested: 0, 0.19, 1.45, 4.83 mg/L	During exposure strong temporary narcotic effect in both sexes **LOAEC $_{rat\ f}$ = 4.83 mg/L** based on observation of narcotic effects
TL2 (1984b), unpublished study report, confidential **Rabbit**; New Zealand White; male/female (5/sex/dose); **acute dermal toxicity study**, occlusive, 24 h**;** protocol similar to EPA OTS 798.1100 Guideline, GLP compliant Test substance: butanone oxime purity: 99.5 % Doses tested: 0.02, 0.2, 2.0 mL/kg bw (18, 185, 1848 mg/kg bw)	**LOAEL$_{rabbit,\ m/f}$ = 185 mg/kg bw** based on significant effects on nervous system (narcosis) **LOEL$_{rabbit,\ m/f}$ = 18 mg/kg bw** based on reversible narcotic effects, occurring during 48 hours following exposure
TL9 (1991), unpublished study report, confidential; Schulze and Derelanko (1993) **Rat**; Sprague-Dawley; male/female (main study: 10/sex/dose; satellite groups: 4/sex/dose) **sub-chronic oral (gavage) toxicity study**, equivalent to OECD TG 408/EU B.26, GLP compliant Test substance: butanone oxime purity: > 99.8 % Doses tested: 0, 40, 125, 400 mg/kg bw/day, exposure duration: 5days/week, 13 weeks	**400 mg/kg bw/d (m/f):** clinical signs: changes (transient and reversible) in neurobehavioral function consistent with CNS depression (hypoactivity, ataxia, impaired aerial righting); dark-coloured urine **NOAEL$_{m/f}$ = 125 mg/kg bw/d** based on neurobehavioral effects

TL19 (1990b), unpublished study report, confidential; Derelanko et al. (2003) **Rabbit**; New Zealand White; female (18/dose); **developmental toxicity study, oral (gavage)**, according to OECD TG 414/ EU B.31, GLP compliant Test substance: butanone oxime purity: > 99 % Doses tested: 0, 8, 14, 24, 40 mg/kg bw, exposure duration: GD6-18 (daily)	**Preliminary study (dose range-finding study):** **≥ 40 mg/kg bw/d:** clinical signs: laboured breathing, decreased activity, few or no faeces **Main study: 40 mg/kg bw/d:** clinical signs: decreased activity, wobbly gait, no faeces, ↓: bw, food consumption; **$LOAEL_f$ = 40 mg/kg bw/d** based on neurobehavioral effects

Single oral doses of ≥ 300 mg/kg bw butanone oxime administered by gavage were found to produce transient and reversible changes in neurobehavioral function consistent with CNS depression, but no evidence of cumulative neurotoxicity was detected (TL8, 1991, unpublished study report, confidential; Schulze and Derelanko 1993). In the acute inhalation toxicity study a strong, transient narcotic effect occurred in both sexes at 4.83 mg/L/4h during the exposure (TL2, 1984a, unpublished study report, confidential). In a dermal acute toxicity study in rabbits butanone oxime produced narcosis at single doses of 185 mg/kg bw and higher. Narcosis was transient at the low dose level of 18 mg/kg bw and higher occurring during the first 48 hours following exposure.

An acute neurotoxicity study and a sub-chronic study both in rats and with oral exposure by gavage revealed transient and reversible functional disturbances in nervous system function consistent with CNS depression. Single oral application of butanone oxime produced significant dose-related decreases in motor activity within one hour after exposure which reached statistically significance at 900 mg/kg bw. Increased ease of cage removal and handling were also noted. Repeated oral application of 300 mg/kg bw or higher for 13 weeks induced transient findings, i.e. impaired gait, aerial righting reflex, and flattened posture. No progressive long-term, irreversible neurotoxic changes were associated with repeated butanone oxime administration for 13 weeks at doses up to 400 mg/kg bw/d. Female rabbits (dams) treated with 40 mg/kg bw/d and higher during the GD6-18 exhibited neurological effects, e.g. decreased activity and unsteady wobbly gait.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

The evidence for specific target organ toxicity through single exposure to butanone oxime was obtained from animal testing. In rats single oral doses of ≥ 300 mg/kg bw butanone oxime administered by gavage produced narcotic effects (TL8, 1991, unpublished study report, confidential; Schulze and Derelanko 1993). In the acute inhalation toxicity study a strong transient narcotic effect occurred in both sexes at 4.83 mg/L/4h (TL2, 1984a, unpublished study report, confidential). In a dermal acute toxicity study in rabbits butanone oxime produced significant defensive or narcotic effects on the CNS at single doses of 185 mg/kg bw and higher, and transient narcotic effects occurring during the first 48 hours following exposure at the low dose level of 18 mg/kg bw. Also in specific investigations transient and reversible functional disturbances in nervous system function consistent with CNS depression were observed in rats after single or repeated oral application of butanone oxime.

In a sub-chronic toxicity study in rats, transient neurobehavioral changes (on cage removal, handling, posture, gait, arousal, salivation, approach response, rearing responses, and aerial righting) were noted immediately after oral dosing with 400 mg/kg bw/d. These changes in neurobehavioral function

were consistent with CNS depression, but no evidence of cumulative or persistent neurotoxicity was detected. A dose of 125 mg/kg bw/d butanone oxime did not induce changes in neurobehavioral function or nervous system structure in rats. In a developmental toxicity study with oral application of butanone oxime, rabbits (dams) showed neurological effects, e.g., decreased activity, wobbly gait, at oral doses of 40 mg/kg bw/d and higher.

Information with respect to toxicity after single exposure, e.g. findings of narcosis, in humans are not available from case reports, epidemiological studies, medical surveillance or national poisons centres.

4.3.2 Comparison with criteria

Specific target organ toxicity, single exposure (STOT SE) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance. These adverse effects produced by a single exposure include consistent and identifiable toxic effects in humans, or, in experimental animals, which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism, and these changes are relevant for human health.

The following applies for the classification as

'STOT-SE Category 3: Transient target organ effects:'

This category only addresses narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria of Categories 1 or 2. Transient target organ effects of Category 3 are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Criteria for classification of substances for narcotic effects are defined in Annex I: 3.8.2.2.2 of CLP. Guidance values are not provided for Category 3 substances.

Classification in Category 3 is primarily based on human data, if available, animal data can be included in the evaluation.

'*The criteria for classifying substances as Category 3 for narcotic effects are:*

(a) Central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgement, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness;

Criteria for narcotic effects observed in animal studies are defined under point

(b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.'

Narcotic effects were observed in several animal studies with different application routes immediately or shortly delayed after administration of butanone oxime. Data from acute oral, inhalation and dermal toxicity testing in rats and rabbits have shown a strong transient narcotic effect in both sexes following single exposure to butanone oxime. In rats significant dose-related decreases in motor activity were observed one hour after single oral dose of 300 mg/kg bw butanone oxime which reached statistically significance at 900 mg/kg bw. In addition increased ease of cage removal and

handling were seen. In rabbits transient narcotic effects occurred during the first 48 hours following exposure by skin at the low dose level of 18 mg/kg bw and higher. During a sub-chronic toxicity study in rats, transient neurobehavioral changes were noted immediately after oral dosing with 400 mg/kg bw/d. In a developmental toxicity study, rabbits (dams) showed neurological effects, e.g. decreased activity, wobbly gait, at the time immediately after application of oral doses of 40 mg/kg bw/d and higher.

The available data from acute oral, inhalation and dermal toxicity and also result from repeated dose toxicity studies have shown clear evidence of transient narcotic effects of butanone oxime in rats and rabbits. Based on these data, butanone oxime meets the criteria for classification and labelling as a specific target organ toxicant (single exposure) of Category 3 for narcotic effects according to CLP (Annex I: 3.8.2.2.2.).

4.3.3 Conclusions on classification and labelling

According to CLP butanone oxime has to be classified as:

STOT-SE 3 and labelled with hazard statement H336: May cause drowsiness or dizziness; with the pictogram "GHS07: Exclamation mark", and with the signal word "Warning".

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

Summary of the Dossier Submitter's proposal

The evidence for specific target organ toxicity following a single exposure to butanone oxime was obtained from animal testing. Information with respect to toxicity after single exposure, e.g. findings of narcosis, in humans are not available from case reports, epidemiological studies, medical surveillance or national poisons centres.

Narcotic effects were observed in several animal studies with different application routes immediately or shortly after administration of butanone oxime. Data from acute oral, inhalation and dermal toxicity testing in rats and rabbits have shown a strong transient narcotic effect in both sexes following single exposure to butanone oxime. In rats significant dose-related decreases in motor activity were observed one hour after a single oral dose of 300 mg/kg bw butanone oxime which reached statistical significance at 900 mg/kg bw. In addition increased ease of cage removal and handling were seen. In rabbits, transient narcotic effects occurred during the first 48 hours following exposure by skin at the low dose level of 18 mg/kg bw and higher. During a sub-chronic toxicity study in rats, transient neurobehavioral changes were noted immediately after oral dosing with 400 mg/kg bw/d. In a developmental toxicity study (Derelanko *et al*. 2003), rabbits (dams) showed neurological effects, e.g. decreased activity, wobbly gait, at the time immediately after application of oral doses of 40 mg/kg bw/d and higher.

The available data from acute oral, inhalation and dermal toxicity and also result from repeated dose toxicity studies have shown clear evidence of transient narcotic effects of butanone oxime in rats and rabbits. Based on these data, the Dossier Submitter concluded

that butanone oxime meets the criteria for classification and labelling as a specific target organ toxicant (single exposure) of Category 3 for narcotic effects.

Although butanone oxime has the potential to damage several organs after a single exposure, including those of the respiratory and blood systems, the Dossier Submitter considered this toxicity specifically as a repeated dose effect. No rationale was provided in relation to the possibility of a classification relating to specific target organ toxicity category 1 or 2.

Comments received during public consultation

One MS was in direct support of the classification of STOT SE 3 for narcotic effects, noting that narcosis is a common effect in laboratory animals for low molecular weight oxime compounds. Another was also in support of the classification but questioned the relevance of the rabbit developmental toxicity study, observing that the behavioural effects observed had occurred at doses producing a high level of mortality. In addition, this MS requested further consideration of the observations of olfactory epithelium degeneration in rats and mice in repeated dose studies. They suggested that if the effects on the respiratory system were sufficiently severe and occurred rapidly after exposure, then classification with STOT SE could be considered.

The Dossier Submitter responded to clarify that there were no data available from the acute toxicity studies on olfactory epithelium degeneration. In their view it was not possible to conclude whether such damage occurred following a single exposure to butanone oxime or if classification with STOT SE for respiratory tract irritation should be considered. They noted that the observed degenerative effects were reversible and not severe in nature. The original epithelium was replaced by repair tissue. Also, the primary site of action was not the respiratory epithelium, suggesting that the mucosal degeneration was not likely due to direct irritation/cytotoxicity with a gradient of severity starting with the most severe effects in the anterior nose regions. In addition, the effects to the olfactory epithelium occurred following inhalation *and* exposure *via* the drinking water. The Dossier Submitter noted that in the drinking water study, vapourisation of test substance could have occurred during water uptake; therefore it was not established that the nasal damage was a systemic effect.

Assessment and comparison with the classification criteria

Transient target organ effects

Narcotic effects

Narcotic effects have not been reported in humans, but there are a number of acute toxicity studies in which signs of narcosis were observed.

In an acute neurotoxicity study, Sprague-Dawley rats (10/sex/dose) received a dose of 0, 100, 300 or 900 mg/kg bw butanone oxime. There were no deaths in any dose group, but from a dose of 300 mg/kg animals were noted to have impaired gait and disturbed aerial righting reflex. These effects were described as transient. At 900 mg/kg bw, statistically significant decreases in motor activity were reported within one hour of exposure.

In an acute inhalation study, F344 rats (5/sex/concentration) were exposed to 0, 0.19, 1.45 and 4.83 mg/L butanone oxime vapour for 4 hours (whole body exposure). No deaths occurred. A temporary, strong narcotic effect was noted in both males and females exposed to the top concentration of 4.83 mg/L. No further details were provided.

In an acute dermal study, butanone oxime was applied to the skin of New Zealand White rabbits (5/sex/dose) at doses of 0, 18, 185 or 1 848 mg/kg bw. At the top dose of 1 858 mg/kg bw, all animals died. Transient narcotic effects were noted from the lowest dose of 18 mg/kg bw during the first 48 hours following exposure. At the next dose of 185 mg/kg bw, these effects were considered significant. No further details were provided.

In a 90-day repeated dose study in Sprague Dawley rats (10/sex/dose), transient neurobehavioral changes were noted in males and females immediately after oral dosing with 400 mg/kg bw butanone oxime. These changes included ease of handling on cage removal, posture, gait, arousal, salivation, rearing responses and aerial righting. No evidence of cumulative or persistent neurotoxicity was noted. No such changes were noted at a dose level of 125 mg/kg bw.

Further evidence of narcosis was presented in a developmental study in New Zealand White rabbits. Clinical signs including decreased activity, laboured breathing, wobbly gait were noted in both the preliminary study and the main study. However, in the preliminary study 2/5 animals died (gestational days 10-11) and in the main study 8/18 animals died (gestational days 11-24). Therefore, 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.

The CLP criteria indicate that for classification for narcotic effects, observations in animal studies may include lethargy, lack of coordination, loss of righting reflex and ataxia. If these are not transient in nature, they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure. Otherwise Category 3 should be considered.

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. Signs of narcosis following a single exposure were noted in two species (rats and rabbits) following oral and inhalation exposure (rats) and dermal exposure (rabbits). These signs included impaired gait, disturbed aerial righting reflex, ataxia and hypoactivity and were all considered transient. In oral studies, narcosis was seen from a dose of 300 mg/kg bw, in inhalation studies from concentrations of 4.83 mg/L and following dermal exposure, effects were observed from a dose of 18 mg/kg bw.

The criteria for classification for specific target organ toxicity after a single exposure have been met. Butanone oxime should be classified for STOT SE 3; H336 (May cause drowsiness or dizziness).

<u>Respiratory irritation</u>

As butanone oxime is irritating to the eyes, there is the possibility that it may also be irritating to the respiratory tract. Respiratory tract irritation has not been reported in humans. Neither single dose nor repeated dose studies in animals show evidence of respiratory tract irritation

i.e. no hyperemia, oedema or inflammation has been observed. There were no reports of significant discomfort of animals following inhalation exposure to butanone oxime. Therefore, no classification for respiratory irritation is necessary.

Target organ toxicity

Respiratory tract

Several repeated dose studies in rats and mice have alluded to the toxicity of butanone oxime to the olfactory epithelium. This has been observed following both repeated dose inhalation and oral studies. The available standard acute toxicity studies, aimed at assessing lethality, do not provide any relevant data to further inform about this toxicity which may occur after only one or very few exposures.

Toxicity to the olfactory epithelium was evident in long-term repeated inhalation exposure toxicity studies conducted in both rats and in mice (see Section: RAC evaluation of specific target organ toxicity - repeated exposure). A further study in male CD-1 mice was conducted specifically to investigate the degenerative and regenerative changes in the olfactory epithelium observed following exposure to butanone oxime. Although a repeated dose study, this also provided an insight to the toxicity of butanone oxime following a short-term exposure. The animals were exposed to 0, 3, 10, 30 or 100 ppm (equivalent to 0, 0.011, 0.036, 0.108 or 0.360 mg/L) butanone oxime for 1, 2, 4, or 13 weeks (6 h/day, 5 days/week) by inhalation with a recovery period of either 4 or 13 weeks (10 mice/group for the full exposure period; 5 mice/group for the interim periods).

At the end of weeks 1, 2, 4 and 13, degeneraton of the olfactory epithelium lining the dorsal meatus was seen in the anterior region of the nasal cavity (turbinates 2-4 only). In a few instances, the olfactory epithelium covering the tips of the nasoturbinal scrolls projecting into the dorsal region of the nasal cavity was also degenerated. Large areas of olfactory epithelium lying laterally and posteriorly were unaffected. In general, approximately 10 % or less of the total olfactory tissue was affected. In several instances, the degenerated olfactory epithelium was re-epithelialised by squamous/squamoid and/or respiratory types of epithelium. Degeneration, which was dose-related in incidence and severity, was seen at 0.108 mg/L after 5 exposures (incidences not provided) and in several mice exposed to 0.036 mg/L after 13 weeks exposures. The incidence and severity of the degeneration present after 5 exposures did not increase with the longer exposures. Recovery was reported to be complete within 4 weeks following exposures at 10 ppm and nearly complete within 13 weeks after exposures at 0.108 and 0.36 mg/L. This recovery involved replacement of the olfactory epithelium with respiratory epithelium and therefore the damage cannot be regarded as being strictly reversible.

Degeneration of the nasal olfactory epithelium was also observed in 90-day studies in which butanone oxime was administered in the drinking water to rats (at doses of 630 mg/kg bw/day and higher) and mice (at doses of 175 mg/kg bw/day and higher). The finding was noted to be of minimal to moderate severity, and in rats, it was observed in the posterior section only (see Section: RAC evaluation of specific target organ toxicity – repeated exposure). Insufficient information is available to determine whether this toxicity to the olfactory tissue occurred systemically after absorption or as a direct effect caused by inadvertent direct exposure in this study.

Both of the two rat acute inhalation studies available, carried out to maximum concentrations of 4.83 mg/L and 10.5 mg/L, respectively, did not show any gross effects to the olfactory epithelium. As microscopic lesions were not investigated, this does not detract from the possibility that butanone oxime does have potential to damage this tissue after a single exposure. No further studies investigated if degeneration of the olfactory epithelium occurred after a single dose, but it was observed after just 5 exposures, and whilst the finding was seen to increase in incidence and severity with increase in dose, it did not progress with time. The nature of the findings are microscopic. The degeneration observed does not lead to any gross observations of tissue damage and studies in mice indicate that it is reversible. Severity gradings ranged from minimal to moderate and there are no studies in which severe damage to the olfactory epithelium has been reported, even at long-term exposures leading to carcinogenicity in other tissues in the lifetime studies in rats and mice.

Overall, although there is some uncertainty whether these findings to the nasal olfactory epithelium can be found after a single exposure, RAC regards them as being indicative of a potential acute effect. As the findings were limited specifically to turbinates 2-4 of the olfactory epithelium and were seen following both inhalation and drinking water exposure to butanone oxime, RAC believes they are a significant target organ effect and classification with STOT SE is appropriate. Effects were observed at exposures as low as 0.108 mg/L in mice (5 exposures) and therefore the criteria for **classification in STOT SE category 1** are met. The hazard statement **H370 (Causes damage to upper respiratory tract)** is appropriate.

4.4 Irritation

4.4.1 Skin irritation

Table 16: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
no specific test method: exposure occlusive over 24 h; observation period: 72 h; 3 non-abraded and 3 abraded skin sites; undiluted test substance; GLP compliant **Rabbit**; New Zealand White (6 animals, sex not specified)	**slightly irritating** to non-abraded and abraded skin of rabbits; PDII (Primary dermal irritation index, according to US CPSC/US OSHA): ca. 1.5 (mean (erythema and oedema)), not fully reversible within 72h; scores reversible at the end of the observation period According to CLP **no classification for skin corrosion/irritation**	In vivo study, reliable with restrictions Test material: butanone oxime, purity: confidential (> 98 %)	TL1 (1978b), unpublished study report, confidential
equivalent to OECD TG 404/EU B.4; no GLP compliance; exposure semi-occlusive over 4h; undiluted test substance **Rabbit**; New Zealand White; sex and no. of animals not reported	**not irritating** following a 4 hour exposure under semi-occlusive conditions to shaved skin According to CLP **no classification for skin corrosion/irritation**	In vivo study, reliable with restrictions Test material: butanone oxime, purity: confidential (> 98 %)	TL3 (1971c), unpublished study report, confidential

4.4.1.1 Non-human information

There are results from two experimental studies in rabbits available. In one study performed by a protocol similar to OECD TG 404/EU B.4, no irritation was observed in rabbits following a 4 hour exposure to butanone oxime under semi-occlusive conditions to the shaved skin. In the other study butanone oxime has been shown to be slightly irritating to the skin of rabbits; the primary irritation index was 1.5. In the available studies butanone oxime was used as test material. The results of experimental studies on skin irritation are summarised in Table 16 (s. there).

4.4.1.2 Human information

No information is available on the skin irritation of butanone oxime in humans.

4.4.1.3 Summary and discussion of skin irritation

The assessment of skin irritation of butanone oxime is based on animal testing. There are no epidemiological studies, clinical studies or case reports available reporting on skin irritation of butanone oxime in humans.

Butanone oxime caused no irritation in rabbits after a 4-hour application time. Butanone oxime has been shown to be slightly irritating to the skin of rabbits after exposure over a period of 24 hours.

4.4.1.4 Comparison with criteria

Evaluation criteria for local effects on the skin are severity of the damage and reversibility. Skin irritation means the production of reversible damage to the skin following the application of a test substance for up to 4 hours. In a tiered approach, emphasis shall be placed upon existing human data, followed by existing animal data, followed by in vitro data and then other sources of information.

The following applies in comparison to

'Skin irritation category: Irritation (Category 2):

(1) Mean score of ≥ 2.3 and ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

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

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

No sign of irritation was observed in a guideline-confirming study on rabbits following a 4-hour application time to butanone oxime. In another study the primary dermal irritation index was 1.5 after exposure over 24 hours, which is below the mean scores that may require classification.

4.4.1.5 Conclusions on classification and labelling

Based on the available data in animals and according to CLP, classification of butanone oxime as Skin Irrit. Cat. 2 is not warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Two studies in rabbits are available to assess skin irritation of butanone oxime. In the first study, no irritation was observed after a 4 hour application time. In the second study, butanone oxime was shown to be slightly irritating to rabbits following a 24 hour exposure period. The DS concluded that the classification criteria were not met and therefore classification of butanone oxime as a skin irritant is not warranted.

Comments received during public consultation

One comment from a MS was received in support of no classification for skin irritation, based on the results of the 2 studies described by the DS.

Additional key elements

In addition to the studies presented in the CLH report, a third study is available in the REACH registration dossier (see the list of additional references not included in the CLH report in the end of the opinion).

In this study, conducted in 1991 according to EPA guidelines and GLP, butanone oxime was applied to the shaven skin of New Zealand White Rabbits (n = 3, sex unknown) occlusively for a period of 4 hours. Animals were then observed for a period of 14 days or until irritation could no longer be observed. Individual animal scores were not provided, but mean scores for erythema and oedema at 24, 48 and 72 h were given.

	Time point				
	24 h	**48 h**	**72 h**	**Mean**	**Reversibility at 14 days**
Erythema	1.7 Max score 2	2 Max score 3	1.7 Max score 3	1.8	Not reversible in 2/3 rats
Oedema	1 Max score 2	2 Max score 3	2 Max score 3	1.7	Not reversible (number of rats not stated)

The results show a mean score for erythema (24, 48 and 72 h) of 1.8, and erythema was not reversible in 2/3 rats at the end of the 14 day observation period. The mean score for oedema (24, 48 and 72 h) was 1.7. This was again found to not be fully reversible in at least one animal by the end of the 14 day observation period. The number of animals for which oedema was observed at the end of the observation period was not clear.

The Registrant concluded that, under CLP, butanone was a category 2 skin irritant in the rabbit.

Assessment and comparison with the classification criteria

Two skin irritation studies in rabbits are available in the CLH report, both with limitations.

In a 1971 study, carried out similarly to OECD guidelines, butanone oxime was applied to the shaved skin of New Zealand White rabbits (numbers and sex unknown) for 4 hours (semi-occlusive). The observation period was not specified. No skin irritation was observed.

In a non-guideline study, dated 1978, butanone oxime was applied to abraded (n = 3) and non-abraded (n = 3) skin of New Zealand White rabbits (occlusive, 24 hour exposure). Erythema and oedema were observed during the 72 hour observation period – findings were not reversible during this time. Scoring was carried out according to the Primary dermal irritation index (mean score of 1.5 for erythema and oedema combined) and is therefore not relevant for classification under CLP.

In addition to the two studies available in the CLH report, a third study was presented in the REACH Registration dossier. This study, dated 1991, appeared to be conducted according to

guidelines and GLP. Butanone oxime was applied to New Zealand White rabbits (n = 3) under occlusive conditions for 4 hours and they were observed for a period of 14 days. Mean scores for erythema and oedema at 24, 48 and 72 hours were provided (scores for erythema = 1.8 and oedema = 1.7). A score of 3 for erythema and oedema was observed in at least one animal at the 48 h and 72 h timepoints. These findings were not reversible within the 14 day observation period.

Conclusion

Butanone oxime has been found to cause irritation to the skin of rabbits, which in some cases, is not reversible within the observation period of 14 days.

As there was no evidence of butanone oxime causing destruction of skin tissue, classification in category 1 (corrosion) is not appropriate.

In order to be classified as a category 2 skin irritant, a substance should have either:

1) a mean value of ≥ 2.3 – ≤ 4.0 for erythema/oedema in at least 2/3 tested animals from gradings at 24, 48 and 72 hours after patch removal; or

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

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

As individual animal scores were not provided to RAC, it is unclear whether the mean scores (24-72 h) observed meet the criteria for classification, however the findings were not reversible in at least 2/3 animals at the end of the 14 day observation period. Therefore, contrary to the CLH report, based on data in the REACH registration database, the **classification as Skin Irritant 2; H315 (Causes skin irritation)** is appropriate.

4.4.2 Eye irritation

Table 17: Summary table of relevant eye irritation study

Method	Results	Remarks	Reference
equivalent to OECD TG 405/EU B.5; GLP compliant undiluted test substance Observation period: 72 h **Rabbit**; New Zealand White; 6 animals, sex not specified	**irreversible effects on the eye** corneal opacity, irititis, conjunctively hyperaemia (score: ≥ 2) in 6/6 animals at 24, 48, and 72 h after exposure; necrosis of the conjunctivae in 2/6 animals, not reversible at the end of observation period According to CLP butanone oxime fulfils the criteria for classification as **Eye Cat 1, H318: Causes serious eye damage**	**Key study** In vivo study Test material: butanone oxime, purity: confidential (> 98 %)	TL1 (1978c), unpublished study report, confidential

4.4.2.1 Non-human information

There are results from an experimental study equivalent to OECD TG 405/EU B.5 performed in 6 rabbits. The test results showed that butanone oxime caused serious eye damage which was not fully reversible. In the available study butanone oxime was used as test material. The results of the experimental study on eye irritation are summarised in Table 17 (see there).

4.4.2.2 Human information

No information is available on serious eye damage/eye irritation of butanone oxime in humans.

4.4.2.3 Summary and discussion of eye irritation

The assessment of eye irritation of butanone oxime is based on animal testing.

The results from the available eye irritation study in rabbits have provided evidence that butanone oxime caused serious eye damage. Corneal opacity, irititis and hyperaemia of the conjunctivae were observed 24, 48, and 72 hours post exposure (average scores 2 or above) in 6/6 animals. Conjunctivae necrosis was observed in 2/6 rabbits which was not reversible at the end of the observation period. Based on these data it is concluded that butanone oxime has the potential to seriously damage the eyes.

Butanone oxime is classified in Annex VI of CLP as Eye Dam. 1, H318.

4.4.2.4 Comparison with criteria

Serious eye damage means the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application. Eye irritation means the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application. In a tiered approach, emphasis shall be placed upon existing human

data, followed by existing animal data, followed by in vitro data and then other sources of information.

Evaluation criteria for local effects on the eye of rabbits are the nature, intensity (severity of the damage) and reversibility of responses. Evaluation takes place separately for cornea, iris and conjunctiva (erythema and swelling).

Substances are allocated to one of the categories within the hazard class, Category 1 (serious eye damage) or Category 2 (eye irritation) as follows:

(a) Category 1 (serious eye damage): substances that have the potential to seriously damage the eyes

(b) Category 2 (eye irritation): substances that have the potential to induce reversible eye irritation

The following applies for classification for substances based on standard animal test data as *'Serious eye damage (Category 1):* A substance that produces:

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

(b) in at least 2 of tested animals, a positive response of
(i) corneal opacity ≥ 3: and/or
(ii) iritis > 1.5

Calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.'

The available eye irritation study in rabbits showed that butanone oxime caused serious damage to the eyes. Butanone oxime caused corneal opacity, irititis and hyperaemia of the conjunctivae. These lesions were observed 24, 48, and 72 hours post exposure (average scores 2 or above) in 6/6 animals. In addition, irreversible effects on the eye were observed in 2/6 rabbits. Conjunctival necrosis was observed in these rabbits which was not reversible at the end of observation period.

4.4.2.5 Conclusions on classification and labelling

Based on the available results, butanone oxime meets the criteria for classification and labelling as 'Irreversible effects on the eye' Category 1, H318 according to CLP, and the legal classification as Eye Dam. 1 is warranted.

According to CLP butanone oxime has to be classified as:

Eye Dam. 1 and labelled with hazard statement H318: Causes serious eye damage; with the pictogram "GHS05: Corrosion", and with the signal word "Danger". The legal classification of butanone oxime as 'Eye Dam. 1' is confirmed.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In a single study, performed in 6 rabbits, butanone oxime caused serious eye damage which was not fully reversible. Based on the results of this study, the DS proposed that butanone oxime meets the criteria for classification for Eye Damage 1; H318.

Comments received during public consultation

Two comments from Member States were received in support of the classification for Eye Dam. 1. One requested further details as to whether the criterion of cornea opacity ≥ 3 was reached. The DS responded that no further details on this were available.

Assessment and comparison with the classification criteria

In an eye irritation study (1978), butanone oxime was applied to one eye of each of 6 New Zealand White rabbits (sex not specified). Corneal opacity, iritis and hyperaemia of the conjunctivae were observed in all 6 rabbits at 24, 48 and 72 h post exposure. Individual scores were not given but the average scores were described as ≥ 2 for 6/6 rabbits. Conjunctival necrosis was observed in 2/6 rabbits; this was not reversible at the end of the observation period of 72 hours (the normal observation period is 21 days).

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

1) 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 (in at least one animal); and/or

2) at least 4/6 animals, a positive response of:
   - Corneal opacity ≥ 3 and/or
   - Iritis > 1.5

Calculated as the mean scores at 24, 48 and 72 hours after installation of the test material.

As individual animal scores weren't given, it is uncertain whether a positive response of ≥ 3 for corneal opacity and/or > 1.5 for iritis were reached, however the finding of necrosis in 2/6 animals indicates a severe reaction which was unlikely to have resolved within 21 days.

Therefore, butanone oxime meets the classification criteria for **irreversible effects on the eye (Category 1); H318 (Causes serious eye damage)**.

4.4.3 Respiratory tract irritation

There are no data on respiratory tract irritation in animals and humans.

4.5 Corrosivity

No information is available on skin corrosion from animal experiments.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 18: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
GPMT according to OECD TG 406/EU B.6, GLP compliant **Guinea pig**; female; Hartley (10/dose)	**Skin sensitising** Induction: 3 % in propylene glycol i.d., 0.3 mL 100 % e.d.; Challenge: 0.2 mL 50 % in propylene glycol e.d. TG: 24h: 9/10, 90 %; 48h: 8/10, 80 % Negative Control: 24h/48h: 0/10 Reliability check: 0.1 % DNCB: 24h/48h: 10/10 According to CLP butanone oxime fulfils the criteria for classification as **Skin Sens. 1B, H317: May cause an allergic skin reaction**	**Key study** Test substance: butanone oxime, purity: confidential (> 98 %)	TL6 (1983), unpublished study report, confidential
Buehler assay according to OECD TG 406/EU B.6, GLP compliant **Guinea pig**; female; Hartley (10/dose; 5/control)	**Skin sensitising** Induction: 25 % in propylene e.d.; Challenge: 5 % in propylene glycol e.d. TG (1. challenge): 24h: 6/10, 60 %; 48h: 5/10, 50 % (2. challenge): 24h: 9/10, 90 %; 48h: 8/10, 80 % Negative Control: 24h/48h: 0/10 Reliability check: 0.1 % DNCB: 24h/48h: 10/10 According to CLP butanone oxime fulfils the criteria for classification as **Skin Sens. 1B, H317: May cause an allergic skin reaction**	**Key study** Test substance: butanone oxime, purity: confidential (> 98 %)	TL20 (1989), unpublished study report, confidential
GPMT according to OECD TG 406/EU B.6, GLP compliant **Guinea pig**; female; Hartley (10/dose)	**Skin sensitising** Induction: 4 % i.d., 100 % e.d.; Challenge: 50 % e.d. TG: 7/10, 70 % No more data According to CLP butanone oxime fulfils the criteria for classification as **Skin Sens. 1, H317: May cause an allergic skin reaction**	Supporting study Specifics not reported, summary results Test substance: butanone oxime, purity: confidential (> 98 %)	TL16 (1989), unpublished study report, confidential
MEST Mouse ear swelling test, no GLP compliance assumed **Mouse**; female; CF-1, (10/ test group, 5/control)	**Skin sensitising** Induction: 50 % in 70 % ethanol e.d.; Challenge: 50 % in 70 % ethanol e.d. TG: 40 % sensitised; 120 % swelling Reliability check: DNCB: 80 % sensitised; 130 % swelling According to CLP butanone oxime fulfils the criteria for classification as **Skin Sens. 1, H317: May cause an allergic skin reaction**	Supporting study Test substance: butanone oxime, purity: 99.98 %	Gad et al. (1986, 1988)
mLLNA according to OECD TG 429/EU B.42, GLP compliant **Mouse**; female; CBA (5/dose)	**Not sensitising** Vehicle: acetone/olive oil (4:1v/v); 50 %, 100 % Stimulation index: 50 %: 1.3; 100 %: 1.0 Reliability check: hexyl cinnamic aldehyde (CAS 101-86-0) According to CLP **no classification for skin sensitisation**	Supporting study Test substance: butanone oxime, purity: confidential (> 98 %)	TL13 (2009), unpublished study report, confidential

4.6.1.1 Non-human information

The skin sensitising potential of butanone oxime was investigated in guinea pigs and mice. Two guinea pig maximisation tests (GPMT) and one Buehler assay according to OECD TG 406/EU B.6 under GLP conditions are available, in addition a local lymph node assay in mice (mLLNA) according to OECD TG 429/EU B.42, and further a mouse ear swelling test (MEST). In the available studies butanone oxime was used as test material. The results of experimental studies on skin sensitisation are summarised in Table 18 (s. there).

Butanone oxime has shown a clear evidence of skin sensitisation in guinea pigs (GPMT and Buehler assay). The results of a mouse ear swelling test (MEST) with butanone oxime have shown a sensitising response of 40 % and a swelling rate of 120 % for the mouse ear. Based on this animal model system a moderate potency for skin sensitisation is determined for butanone oxime. In a standard LLNA in mice, performed under GLP butanone oxime has shown a negative result.

4.6.1.2 Human information

No human data on the sensitising potential of butanone oxime are available.

4.6.1.3 Summary and discussion of skin sensitisation

Data on skin sensitisation of butanone oxime were obtained from animal testing according to the existing testing guidelines.

In two GPMT, and a Buehler assay guinea pigs exhibited positive results. In a MEST a moderate potency for skin sensitisation was determined in mice for butanone oxime. In a standard LLNA in mice butanone oxime concentrations of 50 % and 100 % resulted in stimulation indices (SI) of 1.3 and 1.0, which indicate a negative result in this test system. However, as this result was contradictory to the available reliable assays in guinea pigs and in a test with another mouse strain, more weight was given on the positive tests.

To its allergenic potency and to its relevance to human health butanone oxime was justified as a ‘substance with a solid-based indication of a contact allergenic potential and a substance with the capacity of cross-reactions’ (listed in Category B) by a group of experts including dermatologists from universities, representatives from the chemical industry and from regulatory authorities in Germany (Schlede et al. 2003).

Butanone oxime is currently classified as skin sensitizer category 1 and is listed in Annex VI of CLP as Skin Sens. 1, H317.

4.6.1.4 Comparison with criteria

Skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. Where data are sufficient a refined evaluation according to section 3.4.2.2.1.3 allows the allocation of skin sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other skin sensitisers.

Hazard category and sub-categories for skin sensitisers:

'Category 1: Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

(b) if there are positive results from an appropriate animal test (see specific criteria).'

Based on the available data, butanone oxime is classified as skin sensitizer category 1 and is listed in Annex VI of CLP. The classification is based on positive results from appropriate animal tests: GPMT, Buehler assay, and MEST.

Classification into sub-categories is only allowed if data are sufficient. The available results from animal testing with butanone oxime are sufficient for a refined evaluation allowing the sub-categorisation.

'Sub-category 1A: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.'

'Sub-category 1B: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.'

Comparing with criteria for hazard category and sub-categories for skin sensitizers according to CLP a substance shall be classified for:

Skin sensitisation: Animal test results for Sub-category 1A:

GPMT of ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or

≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or

Buehler assay of ≥ 15 % responding at ≤ 0.2 % topical induction dose or

≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose.

Based on the available data, Sub-category 1A is not appropriate, because the criteria are not fulfilled.

Skin sensitisation: Animal test results for Sub-category 1B:

GPMT of ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or

≥ 30 % responding at > 1.0 % intradermal induction dose or

Buehler assay of ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or

≥ 15 % responding at > 20 % topical induction dose.

In comparison to the given criteria for the hazard category and sub-categories for skin sensitisation according to CLP butanone oxime fulfils the criteria for classification in the hazard class as skin sensitizer Sub-category 1B, H317, because a skin sensitisation response of ≥ 30 % at > 1.0 % intradermal induction dose was observed in the adjuvant type test method (GPMT); and of ≥ 15 % at > 20 % topical induction dose in the non-adjuvant type test method (Buehler assay).

4.6.1.5 Conclusions on classification and labelling

According to CLP butanone oxime has to be classified as:

Skin Sens. 1B and labelled with hazard statement H317: May cause an allergic skin reaction; with the pictogram "GHS07: Exclamation mark", and with the signal word "Warning".

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitising potential of butanone oxime was investigated in guinea pigs and mice. Two guinea pig maximisation tests (GPMT) and one Buehler assay according to OECD TG 406 are available. In addition, there is a local lymph node assay in mice (mLLNA) conducted according to OECD TG 429 and further, a mouse ear swelling test (MEST).

In two GPMT and the Buehler assay, guinea pigs exhibited positive results. In the MEST a moderate potency for skin sensitisation was determined in mice for butanone oxime. In a standard LLNA in mice butanone oxime concentrations of 50 % and 100 % resulted in stimulation indices (SI) of 1.3 and 1.0, which indicate a negative result in this test system. However, as this result was contradictory to the available reliable assays in guinea pigs and in a test with another mouse strain, more weight was given to the positive tests.

Butanone oxime is currently listed in Annex VI of CLP as Skin Sens. 1, H317.

A skin sensitisation response of ≥ 30 % at > 1.0 % intradermal induction dose was observed in the adjuvant type test method (GPMT); and of ≥ 15 % at > 20 % topical induction dose in the non-adjuvant type test method (Buehler assay). Based on the classification criteria for skin sensitisation, the DS proposed that butanone oxime fulfils the criteria for classification as a Skin Sensitiser in sub-category 1B; H317.

Comments received during public consultation

Four comments were received from MS, all in agreement that butanone oxime is a skin sensitiser. However, although one MS was in agreement that sub-categorisation with 1B was justified, the other three MS believed that although the criteria for sub-categorisation with 1B were met, the conditions for sub-categorisation with 1A could not be excluded and therefore, butanone oxime should be classified as Skin Sens. 1 (no sub-categorisation).

Assessment and comparison with the classification criteria

Butanone oxime is currently classified in Category 1 for skin sensitisation in Annex VI of the CLP Regulation.

Guinea Pig Maximisation Tests (GPMTs)

Two tests are available.

The first is dated 1983; only limited details are available. Range-finding was carried out using various concentrations of butanone oxime (0-100 %), applied occlusively for 24 h, in order to find the highest non-irritating concentration (no further details on this were provided). In the main test, 10 female guinea pigs received an intradermal injection of 3 % butanone oxime in propylene glycol for induction. On day 7, a topical induction patch was applied with 0.3 mL of undiluted butanone oxime. On day 21, animals were challenged with 0.2 mL of 50 % butanone oxime in propylene glycol (topical occlusive patch). Twenty-four hours after challenge 9/10 animals showed a positive response. After 48 hours, 8/10 animals showed skin sensitisation reactions. The results in the negative control group showed no reaction at 24 or 48 hours; the positive control group gave the appropriate response.

The results of this study show a 90 % response rate at a 3 % intradermal induction dose, corresponding to classification in sub-category 1B (≥ 30 % responding at > 1 % intradermal induction dose). However, it is not possible to exclude the possibility that the criteria for sub-category 1A could be met on the basis that an intradermal induction of ≤ 1 % was not tested. Given the high number of sensitised animals following induction at 3 %, it is entirely possible that the criteria for 1A could be met.

The second GMPT is dated 1989. Only limited study details are available. Female guinea pigs (10/dose) received an intradermal induction dose of 4 % butanone oxime. Animals were then given a epicutanuous challenge of 50 % butanone oxime. No further details were given. The results of this study showed 7/10 animals with skin sensitisation reactions. It appears that no negative or positive control animals were included.

The results of this study are less reliable in the absence of any data on positive and negative controls. However, a positive result of 70 % following a 3 % intradermal induction concentration was obtained, which fulfils the criteria for classification in sub-category 1B (≥ 30 % responding at > 1 % intradermal induction dose).

Buehler Test

A Buehler test was carried out in female guinea pigs (10/dose group, 5/control group). Range finding tests were carried out in order to ascertain the top non-irritating dose. The induction concentration was 25 % butanone oxime in propylene glycol. Two weeks after the last induction concentration a challenge exposure of 5 % butanone oxime in propylene glycol was applied. A second challenge was performed 1 week after the first challenge, again with 5 % butanone oxime in propylene glycol.

Twenty-four hours after the first challenge, 6/10 animals showed a positive response and after 48 hours, 5/10 animals showed sensitisation. After the second challenge, 9/10 animals showed a sensitisation response after 24 hours and 8/10 showed a response after 48 hours. Negative and positive controls behaved accordingly.

According to the guidance, a positive response of 90 % following an induction concentration of 25 % fulfils the criteria for classification for skin sensitisation category 1B (≥ 15 % responding at > 20 % topical induction dose).

Local lymph node assay (LLNA)

A recent LLNA test is available, carried out according to OECD guidelines and GLP. Female CBA mice (5/dose) were treated with 50 or 100 % butanone oxime in acetone/olive oil (4:1 v/v). The stimulation index was 1.3 at 50 % butanone oxime and 1.0 at 100 % butanone oxime. 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, butanone oxime is not sensitising.

Mouse ear swelling test

Also available is a mouse ear swelling test in which butanone oxime was found to be sensitising. However, as this test is not one of the three recognised and officially accepted animal test methods for skin sensitisation defined by OECD Test Guidelines, it is not considered to add any further weight to this assessment.

Conclusion

The results of two GPMTs and one Buehler test all give solid support for the classification of butanone oxime as a skin sensitiser. The results of a recently and well-performed LLNA with butanone oxime did not show any evidence of skin sensitisation.

Despite the negative result from the LLNA, the weight of evidence provided indicates that butanone oxime should be classified as a skin sensitiser. All three tests providing a positive result indicate classification in sub-category 1B. However, animals were exposed to relatively high concentrations and high responses were seen. In the absence of any data at lower testing concentrations the possibility of sub-classification in category 1A cannot be ruled out. Therefore, according to CLP, classification in category 1 is the default position.

Based on the current evidence base, there is considered to be insufficient information available to justify changing the existing harmonised classification. Therefore the existing

classification **Category 1 for skin sensitisation; H317 (May cause an allergic skin reaction)** should be retained.

4.6.2 Respiratory sensitisation

No data on respiratory sensitisation are available for butanone oxime.

4.7 Repeated dose toxicity

Table 19: Summary table of relevant repeated dose toxicity studies (oral < inhalation)

Method	Results	Remarks	Reference
Oral Sub-chronic 90-day drinking water study equivalent to OECD TG 408/ EU B.26, GLP compliant **Rat**; F344; male/female (10/sex/dose) Doses tested: 0, 312, 625, 1250, 2500, 5000 pm (nominal in water) Exposure duration: daily, ad libitum for 13 weeks	• **Effects indicative of anaemia:** ≥ 100/65 mg/kg bw/d (m/f): ↓ erythrocyte count (-10/-6 %); Hb (-5/-2 %) ≥ 100/30 mg/kg bw/d (m/f): ↑ reticulocyte count (78/25 %) ≥ 175/215 mg/kg bw/d (m/f): ↑ methaemoglobin ≥ 50/65 mg/kg bw/d (m/f): ↑ incidence and severity: haematopoietic cell proliferation in the spleen, haematopoietic proliferation in the bone marrow ≥ 175/215 mg/kg bw/d (m/f): liver: Kupffer cell erythrophagocytosis, haemosiderin pigmentation ≥ 175/120 mg/kg bw/d (m/f): kidney: renal tubular haemosiderin pigmentation **$NOAEL_{m/f}$ = 25/30 mg/kg bw/d** (erythrotoxicity) • **Effects on the respiratory tract:** ≥ 175/215 mg/kg bw/d (m/f): nose: degeneration of the nasal epithelium (olfactory epithelium, posterior nasal section) **$NOAEL_{m/f}$ = 100/120 mg/kg bw/d** (degeneration of the nasal olfac. epithel.) According to CLP: **no classification for STOT-RE** (based on guidance value STOT-RE 2, oral (rat): ≤ 100 mg/kg bw/d)	**Key study** Test substance: butanone oxime, purity: > 99 %	U.S. National Toxicology Program (NTP) (1999)
Oral Sub-chronic 90-day drinking water study equivalent to OECD TG 408/ EU B.26, GLP compliant **Mouse**; B6C3F1; male/female (10/sex/dose) Doses tested: 0, 625, 1250, 2500, 5000, 10000 ppm pm (nominal in water) Exposure duration: daily, ad libitum for 13 weeks	• **Effects indicative of anaemia:** ≥ 755/1010 mg/kg bw/d (m/f): ↑ hematopoietic cell proliferation in spleen, spleen: ↑ weight 1330/3170 mg/kg bw/d (m/f): liver: Kupffer cell erythrophagocytosis indicating intravascular haemolysis and haemosiderin pigmentation; kidney: renal tubule haemosiderin pigmentation **$NOAEL_{m/f}$ = 515/630 mg/kg bw** (extramedullary haematopoiesis, spleen) • **Effects on the urinary bladder epithelium:** m/f: ≥ 515/630 mg/kg bw/d: hyperplasia of the transitional epithelial lining **$NOAEL_{m/f}$ = 200/340 mg/kg bw** (urinary bladder epithelium effects) • **Effects on the nasal olfactory epithelium:** ≥ 755/630 mg/kg bw/d (m/f): degeneration of the olfactory epithelium (minimal to moderate) **$NOAEL_{m/f}$ = 515/340 mg/kg bw** (nasal olfactory epithelium effects) According to CLP: **no classification for STOT-RE** (based on guidance value STOT-RE 2, oral (rat): ≤ 100 mg/kg bw/d)	**Key study** Test substance: butanone oxime, purity: > 99 %	U.S. National Toxicology Program (NTP) (1999)

Oral (gavage) Developmental toxicity study according to OECD TG 414/ EU B.31, GLP compliant **Rat**; Sprague-Dawley; female (preliminary study: 6/dose; main study: 25/dose) Doses tested: 25, 100, 200, 400 mg/kg bw/day in preliminary study. 0, 60, 200, 600 mg/kg bw/day in main study; dose volume: 10 mL Exposure duration: GDs 6-15 (daily)	**Maternal toxicity** *Preliminary study (dose range-finding study)* • **Effects indicative of anaemia:** 400 mg/kg bw/d: clinical signs, transient: wobbly gait, weak body tone, general decreased responsiveness; ↓ bw; blood: reticulocyte (GD16/20: 81/36 %), ↑ methaemoglobin (GD16: 39 %, GD20: 9 %) ≥ 25 mg/kg bw/d: blood: ↑ methaemoglobin (GD16/20: 6/4 %), ↑ reticulocyte count (GD16/20: 18/14 %) ≥ 100 mg/kg bw/d: necropsy: enlarged spleen *Main study* ≥ 200 mg/kg bw/d: clinical signs, transient: wobbly gait, general decreased responsiveness, urine stains ≥ 60 mg/kg bw/d: necropsy: enlarged spleen **$LOAEL_f$ = 25 mg/kg bw/d** (enlarged spleen) According to CLP: **no classification for STOT-RE** (based on equivalent guidance value for studies with exposure shorter than 9 days (i.e. 10 % of the 90 days, STOT-RE 2, oral (rat): ≤ 1000 mg/kg bw/d)	**Key study** Test substance: butanone oxime, purity: > 99 %	TL19 (1990a), unpublished study report, confidential Derelanko et al. (2003)

Oral (gavage) Developmental toxicity study according to OECD TG 414/ EU B.31, GLP compliant **Rabbit**; New Zealand White; female (preliminary study: 5/dose; main study: 18/dose) Doses tested: 0, 10, 20, 40, 80 mg/kg bw/day in preliminary study. 0, 8, 14, 24, 40 mg/kg bw/day in main study; dose volume: 2 mL Exposure duration: GDs 6-18 (daily)	**Maternal toxicity** *Preliminary study (dose range-finding study)* • **Effects indicative of anaemia:** 80 mg/kg bw/d for 2 days (cumulative 160 mg/kg bw) induced mortality: ≤ 48h in 2/5 females; between GD8-10 all 5/5 females died (data considered for classification to acute oral toxicity) clinical signs: dark red or reddish-green coloured urine; necropsy: enlarged spleen, brown discoloured lungs 40 mg/kg bw/d: mortality: 2/5 between GD10-11 ≥ 40 mg/kg bw/d: clinical signs: laboured breathing, decreased activity, few or no faeces, pale ears and/or eyes, eyes dark in colour, brown or reddish coloured fluid in the cage/tray; blood: ↑ reticulocyte (GD13/29: 78/5 %), ↑ methaemoglobin (GD13/29: 42/9 %) 10 mg/kg bw/d: blood: ↑ reticulocyte (GD13/29: 9/5 %), ↑ methaemoglobin (GD13/29: 6/4 %) *Main study* 40 mg/kg bw/d: mortality: 8/18 between GD11-24 clinical signs: decreased activity, wobbly gait, no faeces, greenish or reddish coloured fluid in the cage/tray, urine stains, emaciation, pale eyes and ears, and yellowish coloured crusty material in the nostrils; ↓: bw, food consumption; necropsy: fluid contents in the thoracic cavity, brown discoloration of the lungs, mucoid material attached to the mucosa of the stomach, pale liver, accentuated lobular markings on the liver, urinary bladder with dark red fluid contents and thickened mucosa **$LOAEL_f$ = 10 mg/kg bw/d** (effects indicative of anaemia) According to CLP: **no classification for STOT-RE** (based on equivalent guidance value for studies with exposure shorter than 90 days (STOT-RE 2, oral (rat): 28 day: guidance value ↑ by a factor of 3 → ≤ 300 mg/kg bw/d, 14 days: additionally guidance value ↑ by a factor of 2 → ≤ 600 mg/kg bw/d)	**Key study** Test substance: butanone oxime, purity: > 99 %	TL19 (1990a), unpublished study report, confidential Derelanko et al. (2003)

Inhalation Combined chronic toxicity and carcinogenicity study similar to OECD TG 453/ EU B.33, GLP compliant Interim sacrifice after 3, 12, 18 or 26 months **Rat**; F344; male/female (80/sex/dose) Doses tested: 0, 15, 75 or 374 ppm (nominal) Exposure regimen: vapour (particle size distribution: MMAD: 2.3-2.6 µm, GSD: 2.1-2.8), 6h/d, 5d/wk for 3, 12, 18 and 26 months; whole body	• **Effects indicative of anaemia:** ***3 months:*** 374 ppm (1346 mg/m^3), m/f: blood: ↑: methaemoglobin (1.2 %), MCH (2 %), MCV (6 %), platelets (25 %), leukocytes (6 %), ↓: Hb (4 %), RBC (7 %), MCHC (4 %); ↑ weight, m/f, spleen: (33/33 %); necropsy: spleen: ↑: congestion (m); histopathology: pigment in reticuloendothelial cells (assumed to be haemosiderin), extramedullary haematopoiesis in spleen ***12 months:*** 374 ppm (1346 mg/m^3): blood: males: ↓ Hb, Ht, RBC and platelets, females: ↓ Hb, Ht, RBC and platelets, MCV, MCH; liver: ↑ weight (m), spleen: ↑ weight (m/f, 33 %); necropsy: spleen: congestion (m/f); histopathology: spleen: ↑ extramedullary haematopoiesis (f), ≥ 15 ppm (54 mg/m^3), m/f: spleen: ↑ congestion ≥ 75 ppm (270 mg/m^3): necropsy: spleen: enlarged; histopathology: spleen: pigment in reticuloendothelial cells (hemosiderin) (m) ***18 months:*** ≥ 15 ppm (54 mg/m^3): females: necropsy: spleen: ↑ congestion 374 ppm (1346 mg/m^3): females: histopathology: spleen: ↑ pigment in reticuloendothelial cells, extramedullary haematopoiesis **Reversibility:** haematology parameters, m/f: after 18/26 months • **Liver effects** ***3 months:*** 374 ppm (1346 mg/m^3): ↑ weight, m/f (23/15 %) ***12 months:*** ≥ 75 ppm (270 mg/m^3): basophilic foci and vacuoles in hepatocytes (m), ↓: hyperplasia/proliferation of the biliary duct and peribiliary fibrosis (m/f) ***18 months:*** 374 ppm (1346 mg/m^3), males: basophilic foci and vacuoles in hepatocytes, ↓: hyperplasia/proliferation of the biliary duct and peribiliary fibrosis ***26 months:*** ≥ 15 ppm (54 mg/m^3): ↑ spongiosis hepatis (m) ≥ 75 ppm (270 mg/m^3): ↑ intracytoplasmic vacuoles (m) 374 ppm (1346 mg/m^3): ↑ weight (m: 40 %); histopathology: m/f: ↑ basophilic foci in hepatocytes, spongiosis hepatis; vacuoles • **Effects on the olfac. epithelium in the nasal turbinates** ***12 months:*** ≥ 75 ppm (270 mg/m^3): degeneration on the olfactory epithium in the nasal turbinates characterized by thinning of the layers in the dorsal meatus in turbinate sections 2-4 (m/f) ***18 months:*** 374 ppm (1346 mg/m^3): degeneration of the olfactory epithelium in the dorsal meatus in turbinate sections 2-3 (m/f), 4 (m) ***26 months:*** ≥ 15 ppm (54 mg/m^3): dose-related ↑ degeneration of the olfactory epithelium in the dorsal meatus in turbinate sections 2-4 (m/f) • **Effects on testes:** ***3 months:*** 374 ppm (1346 mg/m^3): ↑ weight (82 %) ***26 months:***374 ppm (1346 mg/m^3): ↑ weight (82 %) without corroborate findings during histopathology **LOAEC$_{sys, m/f}$ = 15 ppm (54 mg/m^3)** based on spleen effects (congestion, increased pigmentation (hemosiderin) in the reticuloendothelial cells, and extramedullary haematopoiesis **LOAEC$_{local, m/f}$ = 15 ppm (54 mg/m^3)** based on effects of the olfactory epithelium in the nasal turbinates According to CLP: **no classification for STOT-RE** (based on guidance value STOT-RE 2, inhalation, vapour: 3 months: ≤ 1 mg/L/6h/d; equivalent guidance value for longer studies: 12 months → ≤ 0.25 mg/L/6h/d; 18 months → ≤ 0.17 mg/L/6h/d; 26 months → ≤ 0.11 mg/L/6h/d)	**Key study** Test substance: butanone oxime, purity: > 99.9 %	Newton et al. (2001); TL18 (1994), unpublished study report, confidential

Inhalation Combined chronic toxicity and carcinogenicity study similar to OECD TG 453/ EU B.33, GLP compliant **Mouse** CD-1; male/female (60/sex/dose) Doses tested: 0, 15, 75 or 374 ppm (nominal) Exposure regimen: vapour (particle size distribution: MMAD: 2.1-2.7 µm, GSD: 2.7-3.4), 6h/d, 5d/wk for 3, 12, 18 and 26 months; whole body	• **Effects indicative of anaemia:** ***12 months:*** ≥ 15 ppm (54 mg/m³): liver: ↑ pigment (hemosiderin) in reticuloendothelial cells ≥ 75 ppm (≥ 274 mg/m³): blood: ↓ MCHC (2.7 %) (f) 374 ppm (1346 mg/m³): blood: ↑: methaemoglobin (m: 0.5 %), ↑: platelets (f: 35 %), ↓: MCHC (f: 3.3 %) ***18 months:*** ≥ 15 ppm (54 mg/m³): liver: ↑ pigment (hemosiderin) in reticuloendothelial cells • **Liver effects:** ***12 months:*** ≥ 15 ppm (54 mg/m³): centrilobular hypertrophy, granulomatous inflammation, and necrosis (m/f) ≥ 75 ppm (≥ 274 mg/m³): centrilobular hepatocellular hypertrophy and necrosis (m) 374 ppm (1346 mg/m³): ↑ relative liver weight (m/f: 12/17 %) ***18 months:*** ≥ 15 ppm (54 mg/m³): centrilobular hypertrophy, granulomatous inflammation, and necrosis (m/f) • **Effects on the olfac. epithelium in the nasal turbinates** ***12 or 18 months:*** ≥ 15 ppm (54 mg/m³): degenerative changes and formation of replacement tissue on the olfactory epithelium in the nasal turbinates (m/f) **$LOAEC_{sys, m/f}$ = 15 ppm (54 mg/m³)** based on effects indicative of anaemia **$LOAEC_{local, m/f}$ = 15 ppm (54 mg/m³)** based on effects of the olfactory epithelium in the nasal turbinates According to CLP: **no classification for STOT-RE** (based on guidance value STOT-RE 2, inhalation, vapour: 3 months: ≤ 1 mg/L/6h/d; equivalent guidance value for longer studies: 12 months → ≤ 0.25 mg/L/6h/d; 18 months → ≤ 0.17 mg/L/6h/d)	**Key study** Test substance: butanone oxime, purity: > 99.9 %	Newton et al. (2001); TL18 (1993), unpublished study report, confidential

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

There are several oral repeated dose toxicity studies of butanone oxime with different time durations available. Butanone oxime was administered by gavage or with the drinking water to rats, mice or rabbits. Data from developmental toxicity studies in rats and rabbits (oral, gavage, according to OECD TG 414/EU B.31; TL19, 1990a,b, unpublished study report, confidential; Derelanko et al. 2003) and from a two-generation study with CD rats (oral, gavage, similar to OECD TG 416/EU B.35; TL17, 1992, unpublished study report, confidential; Tyl et al. 1996) were also evaluated with regards to repeated dose toxicity of butanone oxime. In all studies butanone oxime was used as test material. The results from the NTP studies in rats and mice and from the developmental toxicity studies in rats and rabbits are presented in the summary Table 19 (see there). The results from further experimental studies on repeated dose toxicity after oral administration of butanone oxime are summarised in Table 20.

Table 20: Results from further repeated dose toxicity studies, oral administration of butanone oxime

Reference **Species; strain; sex; method**	**Results**
TL9 (1991), unpublished study report, confidential; Schulze and Derelanko (1993) Sub-chronic, 90-day study, oral (gavage), equivalent to OECD TG 408/EU B.26, GLP compliant **Rat**; Sprague-Dawley; male/female (10/sex/dose) Test substance: butanone oxime purity: > 99.8 % Doses tested: 0, 40, 125, 400 mg/kg bw/day (actual ingested) Exposure duration: daily, 5 days/week, 13 weeks	• **Effects indicative of anaemia:** m/f: ≥ 40 mg/k g bw/d: blood: ↓: RBC count, Hct; ↑: methaemoglobin level, leucocytosis, regenerative anaemia, compensatory reticulocytosis, Heinz body formation, further erythrocytic morphologic changes (not further specified); spleen: ↑ weight **$LOAEL_{m/f}$ = 40 mg/k g bw/d** (effects indicative of anaemia) **$NOAEL_{m/f}$ = 125 mg/kg bw/d** (neurobehavioral effects)
TL1 (1977), unpublished study report, confidential; TL23 (1988), unpublished study report, confidential; TL7 (1990), unpublished study report, confidential Sub-chronic, oral (gavage=, equivalent to OECD TG 408/EU B.26, no GLP compliance **Rat**; Sprague-Dawley; male/female (10/sex/dose) Test substance: butanone oxime purity: confidential (> 98 %) Doses tested: 0, 25, 75, 225 mg/kg bw/day (actual ingested) Exposure duration: daily, 5 days/week, 13 weeks	• **Effects indicative of anaemia:** m/f: ≥ 25 mg/kg bw/d: changes in blood parameters indicative of haemolytic anaemia (data not specified), and compensatory haematopoiesis, extramedullary haematopoiesis in spleen and liver (no more data) **$LOAEL_{m/f}$ = 25 mg/kg bw/d** (haemolytic anaemia and compensatory haematopoiesis)
TL14 (1995a), unpublished study report, confidential Sub-acute, oral (gavage), Method: assessment of peroxisome proliferation, hepatic glutathione and serum testosterone levels, GLP compliant **Rat**, F344; male (15/dose)	Up to 500 mg/kg bw/d: no peroxisome proliferation, no effect on testosterone level ≥ 250 mg/kg bw/d: ↑ hepatic glutathione levels after 14 days, hepatocellular hypertrophy after 14 and 28 days

Reference **Species; strain; sex; method**	**Results**
Test substance: butanone oxime purity: 99.2 % Doses tested: 0, 250,500 mg/kg bw/day (actual ingested) Exposure duration. Daily for 7, 14 or 28 days	
TL12 (1996), unpublished study report, confidential Sub-acute, oral (gavage), Japanese guideline, similar to OECD TG 407/EU B.7, GLP compliant **Rat**; Crj: CD(SD); male/female (7/sex/dose) Test substance: butanone oxime purity: confidential (> 98 %) Doses tested: 0, 4, 20, 100 mg/kg bw/day (actual ingested) Exposure duration: daily for 28 days, recovery period of 14 days	• **Effects indicative of anaemia:** ≥ 20 mg/kg bw/d: blood: ↑ reticulocyte ratio (m/f), ↑ platelet count (f); ↓ RBC count, Hct, Hb (f) 100 mg/kg bw (m/f): spleen: ↑ abs/rel organ weight, hypertrophy, congestion, hemosiderin granules; liver: Kupffer cells: ↑ hemosiderin granules; liver and spleen: extramedullary haematopoiesis; kidney: lipofuscin-like substance in tubular epithelium; reversibility of the most changes at the end of recovery (not further specified) **$LOAEL_{m/f}$ = 20 mg/kg bw/d** (effects on blood parameters indicative of anaemia) **$NOAEL_{m/f}$ = 4 mg/kg bw/d** (effects on blood parameters indicative of anaemia)

Reference Species; strain; sex; method	Results
TL17 (1992), unpublished study report, confidential; Tyl et al. (1996) Two-generation study, oral (gavage); EPA guideline with modifications, similar to OECD TG 416/EU B.35; GLP compliant **Rat**; CD Sprague-Dawley (Crl:CD[SD]BR) VAF/Plus); male/female (30/sex/dose; at least 20 pregnant females/group) Test substance: butanone oxime purity: > 99 % Doses tested: 0, 10, 100, 200 mg/kg bw/day; dosing volume: 2.0 ml/kg bw/day Exposure regimen: F0 generation: starting from 8 wk of age during 10 wk pre-mating, 3 wk mating period (continued dosing). F1 generation: starting from 11 wk of age in the same regime. (5 days/week);	• **Effects indicative of anaemia:** **200 mg/kg bw/d: mortality: 4/30 (13.3 %) F0m, 11/30 (36.7 %) F0f, 15/30 (50 %) in F1m, 8/30 (26.7%) in Flf** **disturbance of general behaviour: tremors, salivation, slow respiration, mouth breathing, lethargy, staggering, and rooting in bedding post dosing in F0m, tremors, ataxia, and convulsions (only in moribund animals), stupor, abnormal respiration (audible, irregular, raspy, laboured), dyspnoea, dehydration, excessive urination, bright yellow urine, and rooting in bedding in F0f, tremors, audible breathing, and rooting in bedding in Flm, and lethargy, abnormal respiration (laboured, gasping, and raspy), cyanosis, and rooting in bedding in Flf** **blood: consistent picture of anaemia in both sexes in both generations: ↓: RBC count, Hb, Hct, ↑: MCV, MCH, WBC count (no change in differential counts) F0m/f + Flm/f, methaemoglobin in F0m + Flm** **necropsy: ↑: abs. and rel. weights of spleen in F0m/f/Flm/f, rel. weights of liver in Flm + F0f/Flf** **histopathology: spleen: congestion, and in spleen and liver: extramedullary haematopoiesis and haemosiderosis in F0m/f + Flm/f** **100 mg/kg bw/d:** **clinical signs of toxicity: F0m: lethargy, staggering, and rooting in bedding; F0f: weaving, tremors, and rooting in bedding; Flm: slight dehydration, audible breathing, and rooting in bedding; and Flf: laboured breathing** **blood: consistent picture of anaemia in both sexes in both generations: F0m + F1m: ↓ RBC count (26/31 %), ↓ Hb (9/14 %), F0f + F1f: ↓ RBC count (16/25 %), ↓ Hb (8/11 %), ↑:WBC (no change in differential counts) in F0m, Flm + F0m:** **↑ methaemoglobin (82%)** **necropsy: ↑ abs. and rel. weights of spleen** **histopathology: in spleen and liver extramedullary haematopoiesis and haemosiderosis in F0m/f + Flm/f** **10 mg/kg bw/d:** **clinical signs of toxicity: F0m +F1m: rooting in bedding** **blood: F0m: ↓ RBC count (10 %), Hb (6 %)** **necropsy: F0m: dark spleens (5/30)** **histopathology: spleen and liver extramedullary haematopoiesis and haemosiderosis in F0m/f + Flm/f** **LOAELm/f = 10 mg/kg bw/d (effects indicative of anaemia and neurobehavioral effects)**

4.7.1.2 Repeated dose toxicity: inhalation

For the inhalation route of exposure five repeated dose toxicity studies with butanone oxime are available. The study results from the combined chronic toxicity/carcinogenicity studies in rats and mice (according to OECD TG 453/EU B.33) were also evaluated regarding non-neoplastic effects. The results from these studies in rats and mice are presented in the summary Table 19 (see there). In all studies butanone oxime was used as test material. The results from further experimental studies on repeated dose toxicity following inhalation route of exposure are summarised in Table 21.

Table 21: Repeated dose toxicity studies, inhalation exposure to butanone oxime

Reference Species; strain; sex; method	Results
TL4 (1990), unpublished study report, confidential Sub-acute, inhalation (vapour), whole body; similar to OECD TG 412/ EU B.8; GLP compliant **Rat;** F344; male/female; Test substance: butanone oxime purity: 99.9 % Doses tested: 0, 30, 101, 340 ppm (nominal) Exposure duration: 6h/d, 5d/wk, 4 weeks	• **Effects indicative of anaemia:** 404 ppm (1440 mg/m³/6h/d) (m/f): blood: ↓ (10 %): Hb, Hct, RBC, MCHC; ↑: methaemoglobin (0.5 %), reticulocytes (threefold), platelets (30 %), leukocytes (13 %); liver and spleen: ↑ weights (30 %), no related histological effects in these organs **$LOAEC_{sys, m/f}$ = 102 ppm (360 mg/m³/6h/d)** (effects on blood parameters) **$NOAEC_{sys, m/f}$ = 25 ppm (90 mg/m³)** (effects on blood parameters) **$NOAEC_{local, m/f}$ = 404 ppm (1440 mg/m³/6h/d)**
TL4 (1990), unpublished study report, confidential Sub-acute, inhalation (vapour), whole body; equivalent to OECD TG 412/EU B.8, GLP compliant **Mouse;** CD-1; male/female; Test substance: butanone oxime purity: 99.9 % Doses tested: 30, 101, 341 ppm (nominal) Exposure duration: 6h/d, 5d/wk, 4 weeks	• **Effects indicative of anaemia:** 400 ppm (1440 mg/m³/6h/d) (m/f): blood: ↑: methaemoglobin (1-2 %), spleen: ↑ weight (30 %), no histology data **$NOAEC_{sys}$ = 102 ppm (360 mg/m³/6h/d)** (effects on blood parameters) **$NOAEC_{local}$ =** no data available
TL14 (1995b), unpublished study report, confidential; Newton et al. (2002) Effects on the olfactory epithelium and recovery were assessed, inhalation (vapour), whole body, equivalent to OECD TG 413/EU B.29, GLP compliant **Mouse;** CD-1; male (Main study: 10/dose/interval; satellite groups: 5/dose/interval) Test substance: butanone oxime purity: 99.2 %	• **Effects on the nasal olfactory epithelium:** ≥ 10 ppm (36 mg/m³/6h/d) (m): After 1, 2, 4, 13 wk: nasal cavity: 10 % of the total olfactory tissue affected, degeneration of the olfactory epithelium in dorsal meatus of the anterior region dose-related ↑ in incidence and severity: ≥ 30 ppm after 1 wk, replacement by squamous/squamoid and/or respiratory epithelium within prolonged recovery of 13 wk; ≥ 10 ppm after exposure for 13 wk, full recovers within 4 wk **$LOAEC_{local}$ = 30 ppm (108 mg/m³) after 1 wk (30 total hours of exposure)** (based on degeneration of the olfactory epithelium in the nasal cavity) **$NOAEC_{local}$ = 3 ppm (10.8 mg/m³/6h/d)** (based on degeneration of the olfactory epithelium in the nasal cavity)

Doses tested: 0.3, 10, 30, 100 ppm (10.8, 36, 108, 360 mg/m3) (analytical conc.) Exposure duration: 6h/d, 5d/wk, for 1, 2, 4, 13 wks, recovery period of 4 or 13 wk, microscopy limited to nasal turbinates;	

4.7.1.3 Repeated dose toxicity: dermal

No information is available in experimental animals.

4.7.1.4 Repeated dose toxicity: other routes

No information is available.

4.7.1.5 Human information

No information is available.

4.7.1.6 Other relevant information

No information is available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The assessment of target organ toxicity through repeated exposure to butanone oxime is based on animal testing. No information with respect to repeated dose toxicity of butanone oxime in humans is available.

Repeated dose toxicity studies on butanone oxime have been conducted in rats and mice using oral application and inhalation. The information on health effects after long-term repeated exposure of butanone oxime by inhalation was complemented by the non-neoplastic results from the combined chronic toxicity and carcinogenicity studies in rats and mice. In addition the results of the oral developmental toxicity studies in rats and rabbits and of a two-generation toxicity study in rats were considered for the evaluation of the specific target organ toxicity (repeated exposure) of butanone oxime.

Oral route: Gavage and drinking water studies with durations of 4 and 13 weeks have been conducted with rats, and one 13-week drinking water study with mice. The maternal toxicity data from developmental toxicity studies using rats and rabbits and the toxicity data of adult rats from repeated oral exposure by gavage observed from a two-generation toxicity study are also included in the assessment of butanone oxime toxicity.

The major target of butanone oxime toxicity was the haematopoietic system (blood) of rats, mice and rabbits. Further lesions observed compromise neurobehavioral effects in rats and rabbits,

degeneration of the nasal olfactory epithelium in rats and mice and hyperplasia of the urinary bladder transitional epithelium in mice.

Butanone oxime caused dose-related increased **effects on blood** parameters indicative of haemolytic anaemia and compensatory medullary haematopoiesis, as well as extramedullary haematopoiesis in the spleen and liver. In studies with rats and mice, the effects on the blood increased regarding incidence and severity were observed at doses ≥ 10 mg/kg bw/d, serious effects were seen in male and female rats at ≥ 175/215 mg/kg bw/d and in male and female mice at ≥ 755/1010 mg/kg bw/d. The effects were methaemoglobinaemia, formation of Heinz bodies, increased reticulocyte count, increased incidences of haematopoietic proliferation in the bone marrow, regenerative haematopoietic cell proliferation in the spleen and liver, liver Kupffer cell erythrophagocytosis and haemosiderin pigmentation, as well as renal tubule haemosiderin pigmentation.

In developmental toxicity studies in rats and rabbits, oral administration of butanone oxime to dams by gavage produced clear evidence of maternal toxicity in both species which showed effects indicative of anaemia. The effects on the blood occurred at ≥ 25 mg/kg bw/d in rats and at ≥ 10 mg/kg bw/d in rabbits. Pregnant animals may show particular sensitivity as mortalities were not seen in non-pregnant rats at 25 mg/kg bw/d. In the two-generation toxicity study in rats oral (gavage) administration of 10 mg/kg bw/d and higher induced reduced red blood cell counts and haemoglobin concentration in F0 males associated with extramedullary haematopoiesis and haemosiderosis in liver and spleen. Findings of extramedullary haematopoiesis and haemosiderosis in liver and spleen, unaccompanied by any other indications of blood toxicity were also seen in Fl male and female rats receiving 10 mg/kg bw/d. Based on the haematology and microscopic findings in spleen and liver an increased anaemia response with a clear dose relation is suggested. Increased spleen weights and splenic and hepatic extramedullary haematopoiesis (haematopoietic cell proliferation) and haemosiderosis (pigment deposition from haemoglobin breakdown products) observed at ≥ 100 mg/kg bw/d (cut off value for classification as STOT-RE2) were consistent with haemolytic anaemia and compensatory erythropoiesis. The effects at this dose levels (≥ 100 mg/kg bw/d) were accompanied by reduced body weight and weight gain, reduced feed consumption, and clinical signs of toxicity (cyanosis).

Overall, the lowest oral $LOAEL_{sys}$ for adverse effects on the blood of butanone oxime was 10 mg/kg bw/d, based on reduced red blood cell counts and haemoglobin concentration associated with extramedullary haematopoiesis and haemosiderosis in liver and spleen of adult male and female rats observed in a two-generation reproduction study (similar to OECD TG 416/EU B.35; TL17, 1992, unpublished study report, confidential; Tyl et al. 1996). For short-term exposures the lowest oral $LOAEL_{sys}$ of butanone oxime was also 10 mg/kg bw/d, based on signs indicative of anaemia in adult female rabbits observed in a range-finding developmental study (according to OECD TG 414/EU B.31; TL19, 1990b, unpublished study report, confidential; Derelanko et al. 2003).

In a sub-chronic study in rats, **transient neurobehavioral changes** (cage removal, posture, impaired gait, arousal, salivation, approach response, rearing responses, and disturbed aerial righting reflex) were noted immediately after dosing with 400 mg/kg bw/d. These changes in neurobehavioral function were consistent with CNS depression, but no evidence of cumulative neurotoxicity was detected. A dose of 125 mg/kg bw/d butanone oxime did not induce changes in neurobehavioral function or nervous system structure in rats. Female rabbits (dams) treated with 40 mg/kg bw/d and higher during the GD6-18 exhibited neurological effects, e.g. decreased activity and wobbly gait. For neurobehavioral effects of butanone oxime a $NOAEL_{sys}$ of 125 mg/kg bw/d was derived in male and female Sprague-Dawley rats from a sub-chronic toxicity study (equivalent to OECD TG 408/ EU B.26; TL9, 1991, unpublished study report, confidential; Schulze and Derelanko 1993).

Degenerative effects on the olfactory epithelium in the nasal turbinates were noted in the sub-chronic drinking water studies in male and female rats at ≥ 2500 ppm (≥ 175/215 mg/kg bw/d) and in male mice at ≥ 5000 ppm (≥ 755 mg/kg bw/d) and in female mice at ≥ 2500 ppm (≥ 630 mg/kg bw/d). For butanone oxime a NOAEL of 100 mg/kg bw/d for effects on the nasal olfactory epithelium was determined in a sub-chronic drinking water study (equivalent to OECD TG 408/EU B.26) with male and female F344 rats (NTP 1999).

Hyperplasia of the urinary bladder transitional epithelium occurred in exposed male and female mice via drinking water for 13 weeks at ≥ 2500 ppm (≥ 515/630 mg/kg bw/d). A $NOAEL_{sys}$ for changes in the urinary bladder (hyperplasia of the transitional epithelium associated with inflammatory reactions) of butanone oxime was established in a sub-chronic drinking water study (equivalent to OECD TG 408/EU B.26) at 110 mg/kg bw/d in male mice and at 340 mg/kg bw/d in female mice (NTP 1999).

Inhalation: Repeated exposure of rats and mice to butanone oxime by inhalation produced methaemoglobin formation, haemolytic anaemia including compensatory regenerative cell proliferation of haematopoietic cells and spleen haemosiderosis, non-neoplastic liver effects (rats: basophilic foci and hepatocyte vacuoles; mice: hypertrophy and necrosis) increased weight of the testes (rats only), and degenerative changes on the olfactory epithelium in the nasal turbinates primarily in the dorsal meatus followed by formation of replacement tissue.

Effects on the blood were observed in sub-acute, sub-chronic and chronic studies in rats and mice. In a sub-acute study in rats butanone oxime caused effects on blood parameters including increased levels of methaemoglobin at concentrations of ≥ 102 ppm (≥ 360 mg/m^3/6h/d) (TL4, 1990, unpublished study report, confidential). A NOAEC of 25 ppm (90 mg/m^3) was derived for effects on the blood. In the combined chronic toxicity and carcinogenicity study in rats (with interim analysis, sacrifices at 3, 12, and 18 months), similar effects on blood parameters were seen at 374 ppm (1346 mg/m^3) after exposures of 3 or 12 months in males and females. Spleen effects (increased organ weight, extramedullary haematopoiesis, and haemosiderosis) occurred at the same concentration and duration of exposure. No effects on blood parameters and no findings in the spleen were noted after 18 months in males or after 26 months in both sexes. In mice, a 4-week inhalation toxicity study showed a slight increase in methaemoglobin levels as well as increased spleen weights at 400 ppm (1440 mg/m^3) and a NOAEC was derived at 100 ppm (360 mg/m^3). At the 12 months sacrifice of an 18 months combined chronic toxicity and carcinogenicity study in mice the effects of butanone oxime on the blood occurred less clear than in rats. A slight methaemoglobin formation was noted in males at 374 ppm (1346 mg/m^3). Methaemoglobin did not appear to be formed in females, but there was a significant increase in platelets (35 %) in the 374 ppm (1346 mg/m^3) group and a significant decrease in mean corpuscular haemoglobin concentration (MCHC) at 76 ppm (2.7 %) and 374 ppm (3.3 %). At termination of the study, no indications of any treatment-related effects on the differential leukocyte count or erythrocyte morphology in either male or female mice were observed. The lowest LOAEC value for haematotoxic effects of butanone oxime was established in rats and mice at 15 ppm (54 mg/m^3) derived from combined chronic toxicity and carcinogenicity studies (similar to OECD TG 453/ EU B.33; 26/18 months study, whole body inhalation, 6h/d, 5d/wk) in both species (Newton et al. 2001; TL18, 1994, 1993, unpublished study reports, confidential).

Liver effects were observed in rats and mice on a dose-related manner in the life-time studies (combined chronic toxicity and carcinogenicity studies, inhalation). The liver changes, indicating hepatotoxicity, included increased incidences of basophilic foci and vacuoles in the hepatocytes of male rats exposed at 75 ppm (270 mg/m^3) and in both sexes exposed at 374 ppm (1346 mg/m^3). Liver effects were seen in mice as increases in centrilobular hypertrophy and necrosis and were noted at 15

ppm (54 mg/m^3) and higher. Accordingly, the LOAEC for systemic effects was derived at 15 ppm (54 mg/m^3) for mice based on the observed liver effects.

As further finding in the combined chronic toxicity and carcinogenicity study **enlarged testes** were seen in male rats exposed at 75 and 374 ppm (270 and 1346 mg/m^3), which did not correlate with any microscopic findings.

Effects on the respiratory system were reported from the inhalation combined chronic toxicity and carcinogenicity studies in rats and mice (similar to OECD TG 453/EU B.33, whole body exposure, 6h/d, 5d/wk). Mice appear to be more susceptible to the nasal effects of butanone oxime than rats. In the nasal turbinates degenerative effect on the olfactory epithelium of the nasal turbinates was noted at all tested exposure concentrations (≥ 15 ppm; equivalent to ≥ 54 mg/m^3). 15 ppm was derived as the lowest LOAEC for effects on the respiratory tract in the mouse observed both after 12 and 18 months of exposure (Newton et al. 2001; TL18 1993, unpublished study report, confidential).

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT-RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT-RE according to CLP Regulation

The evaluation of target organ toxicity through repeated exposure to butanone oxime is based on animal tests conforming to internationally agreed test guidelines. There were studies in rats, mice and rabbits. No information is available on toxicity after repeated exposure to butanone oxime in humans.

Dose-related toxic effects were observed in rats and mice in studies with different testing time periods for both examined routes (oral, inhalation), and were also noted in the developmental toxicity studies in rats and rabbits, and in a two-generation toxicity study in rats given butanone oxime by oral application.

Target organs of toxicity after repeated oral administration of butanone oxime are the haematopoietic system, the nervous system, the liver and the urinary bladder in experimental animals. Effects on the nasal olfactory epithelium were seen after exposure by the oral route and inhalation, thus considered to be a systemic effect.

Haemolytic anaemia was the main toxic effect corresponding with decreases in red blood cell parameters (RBC count, Hb, Hct), damaged erythrocytes (Heinz bodies), methaemoglobinaemia, breakdown product of haemoglobin, and increased degrees of deposited haemosiderin in spleen, liver and kidney, and extramedullary haematopoiesis in spleen and liver, and clinical signs (paleness, cyanosis). The effects on the erythrocytes were generally less severe with the inhalation exposure. The rabbit and the rat appeared to be more sensitive than the mouse to the haemolytic effects of butanone oxime.

In rats anaemic blood effects were observed in sub-chronic oral toxicity studies at doses of ≥ 25 mg/kg bw/d and in a two-generation toxicity study at ≥ 10 mg/kg bw/d. Haematotoxicity was more pronounce in male and female rats at ≥ 175/215 mg/kg bw/d and in mice at doses of ≥ 755/1010 mg/kg bw/d after a sub-chronic exposure. In a two-generation toxicity study in rats, effects on the blood indicative of haemolytic anaemia with concomitant extramedullary haematopoiesis and haemosiderosis in liver and spleen (and increased spleen weights) was observed at 100 mg/kg bw/d and higher.

In developmental studies in rats and rabbits, oral administration by gavage of butanone oxime to dams produced clear evidence of maternal toxicity in both species. Effects on the blood occurred at ≥ 25 mg/kg bw/d in rats and at ≥ 10 mg/kg bw/d in rabbits. Mortalities that occurred in pregnant rabbits after treatment with two oral doses of 80 mg/kg bw/d butanone oxime during the gestation phase, i.e. methaemoglobin formation within the first 48 hours, were covered by classification for acute oral toxicity.

The lowest LOAEC value for effects of butanone oxime indicative of anaemia was established in rats and mice exposed by inhalation at 15 ppm (54 mg/m^3) derived from combined chronic toxicity and carcinogenicity studies (similar to OECD TG 453/EU B.33) in both species (Newton et al. 2001; TL18, 1994, 1993, unpublished study reports, confidential). The red blood cell reduction was at least partly compensated by increased erythrocyte production resulting in release of increased reticulocytes to the blood stream. With increasing demand on the regenerative activity by the bone marrow, extramedullary erythropoiesis was seen mainly in the spleen and the liver. The degree of anaemia was not progressive with long-life repeated inhalation exposure. This development was primary driven by this compensatory erythropoiesis. In the combined chronic toxicity and carcinogenicity study in rats, adaptation by continuous excitation of erythrocyte production was evident with values for most of the erythrocyte parameters being similar between butanone oxime-exposed and control male rats after 18 months of exposure and for females by study termination after 26 months. It is to note that as a consequence of an elevated decompensation of damaged erythrocytes that persistent distress in the liver and the spleen may occur.

Effects on the nasal olfactory epithelium were seen after exposure by the oral route and inhalation, thus considered to be a systemic effect. In sub-chronic drinking water studies (equivalent to OECD TG 408/EU B.26) in male and female rats effects on the nasal olfactory epithelium were seen at ≥ 2500 ppm (≥ 175/215 mg/kg bw/d), and in male mice at ≥ 5000 ppm (≥ 755 mg/kg bw/d) and in female mice at ≥ 2500 ppm (≥ 630 mg/kg bw/d).

In combined chronic toxicity and carcinogenicity studies (similar to OECD TG 453/EU B.33, whole body exposure, 6h/d, 5d/wk) repeated exposure by inhalation to butanone oxime of rats and mice caused effects on the olfactory epithelium of the nasal turbinates at all tested exposure concentrations (≥ 15 ppm; equivalent to ≥ 54 mg/m^3).

In the sub-chronic studies transient neurobehavioral changes were noted immediately after oral application of rats with 400 mg/kg bw/d and in rabbits with ≥ 40 mg/kg bw/d. No changes in neurobehavioral function or nervous system structure was noted in rats at dose level of 125 mg/kg bw/d butanone oxime (see 4.3 Specific target organ toxicity – single exposure (STOT SE)).

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT-RE

Target organ toxicity, repeated exposure (STOT-RE) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, reversible and irreversible, immediate and/or delayed are included.

Classification of substances as specific target organ toxicants following repeated exposure is based on the weight of all evidence available, including the use of recommended guidance values which

take into account the duration of exposure and the dose/concentration which produced the effect(s), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed. STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more pronounced or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health.

Categories for specific target organ toxicity-repeated exposure:

'*Category 1:* Substances that have pronounced 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 (repeated exposure) on the basis of:

- Reliable and good quality evidence from human cases or epidemiological studies, or
- Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. '

Thus classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur within the guidance value ranges according to CLP (Annex I, Part 3, Table 3.9.2, Guidance values to assist in Category 1 classification):

Oral (rat): $C \leq 10$ mg/kg bw/d

Inhalation (vapour, rat): $C \leq 0.2$ mg/L/6h/d.'

No information is available on toxicity after repeated exposure to butanone oxime in humans. Studies in rats, mice and rabbits indicate that the haematological and neurological systems are relevant targets of the toxicity of butanone oxime. However, no significant/severe toxic effects from both the oral and inhalation toxicity studies were observed at dose levels approximately equal to the STOT-RE 1 cut-offs according to CLP (guidance values in Annex I, Part 3, Table 3.9.2).

'*Category 2:* Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in Category 2 for target organ toxicity (repeated exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentration. Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur within the guidance value ranges according to CLP (Annex I, Part 3, Table 3.9.3, Guidance values to assist in Category 2 classification):

Oral (rat): $10 < C \leq 100$ mg/kg bw/d

Inhalation (vapour, rat): $0.2 < C \leq 1.0$ mg/L/6h/d.'

The haematological and neurological systems and the respiratory tract are relevant targets of the toxicity of butanone oxime.

Repeated dose toxicity studies with butanone oxime in rats, mice and rabbits showed haematotoxicity such as anaemia after exposure by oral application or inhalation. The observations of haemotoxic effects caused by butanone oxime are considered represent a borderline case regarding classification for target organ toxicity arising from a repeated exposure. There is no doubt that butanone oxime produced significant health effects after repeated exposure. The effects of butanone oxime on the blood observed after repeated exposure by both the oral and the inhalation routes are considered as 'adverse' and at the high dose levels they can equally be considered as 'severe'. Anaemic findings and secondary effects observed in rats after repeated oral administration of 100 mg/kg bw/d and higher in a two-generation toxicity study and at the highest concentration tested of 374 ppm (1346 mg/m^3) in a 2-year inhalation combined chronic toxicity and carcinogenicity study were not severe enough to justify classification. There was a tendency of reversibility of most of the disturbed erythrocyte parameters in male and female rats at termination of the combined chronic toxicity and carcinogenicity study. The observed increase in haemosiderosis in the spleen, liver or kidney was not combined with severe morphological changes like necrosis, fibrosis or cirrhosis. In conclusion, the effects observed from both the oral and inhalation toxicity studies at dose levels approximately equal to the STOT-RE 2 cut-offs according to CLP (Annex I, Part 3, guidance values: oral (rat): $10 < C \leq 100$ mg/kg bw/d; inhalation (vapour, rat): $0.2 < C \leq 1.0$ mg/L/6h/d) are not considered as significant toxic effects according to the CLP criteria (CLP Guidance, 3.9.2.5.2. Haemotoxicity).

Effects on the nasal olfactory epithelium were seen in rats and mice after exposure to butanone oxime by the oral route and inhalation. Signs of degenerative effects on the nasal olfactory epithelium were noted in male and female rats at doses of ≥ 2500 ppm (≥ 175/215 mg/kg bw/d), and in male mice of ≥ 5000 ppm (≥ 755 mg/kg bw/d) and in female mice of ≥ 2500 ppm (≥ 630 mg/kg bw/d) in sub-chronic drinking water studies which were all above the STOT-RE 2 cut-offs according to CLP (Annex I, Part 3, guidance values: oral (rat): $10 < C \leq 100$ mg/kg bw/d). After long-life repeated exposure to butanone oxime by inhalation effects on the olfactory epithelium of the nasal turbinates were noted in rats and mice at all concentration tested (≥ 15 ppm, equivalent to 0.054 mg/L/6h/d). Findings on the nasal olfactory epithelium observed in rats and mice up to the highest concentration tested of 374 ppm (1.346 mg/L/6h/d) were not severe enough to justify classification. It is concluded that butanone oxime does not fulfil the criteria for classification as STOT-RE according to CLP (STOT-RE 2 cut-offs according to Annex I, Part 3, guidance values: inhalation, vapour, rat: $0.2 < C \leq 1.0$ mg/L/6h/d (3 months); equivalent guidance value for longer studies: ≤ 0.17 mg/L/6h/d (18 months); ≤ 0.11 mg/L/6h/d (26 months).

In the sub-chronic studies (90-day studies) transient neurobehavioral changes were noted immediately after oral application of rats with 400 mg/kg bw/d and in rabbits with ≥ 40 mg/kg bw/d. No changes in neurobehavioral function or nervous system structure was noted in rats at dose level of 125 mg/kg bw/d butanone oxime. Based on these available data, butanone oxime does not fulfil the criteria for classification for target organ toxicity arising from a repeated oral exposure according to CLP (STOT-RE 2: oral (rat): $10 < C \leq 100$ mg/kg bw/d). However these data are considered for the classification of butanone oxime on specific, non-lethal target organ toxicity arising from single exposure (STOT SE 3). For more details see Section '4.3 Specific target organ toxicity – single exposure (STOT SE)'.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT-RE

There is no concern for specific target organ toxicity arising from a repeated oral or inhalation exposure to butanone oxime based on the available data.

According to CLP butanone oxime has not to be classified as STOT-RE.

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

Summary of the Dossier Submitter's proposal

The evaluation of target organ toxicity through repeated exposure to butanone oxime is based on animal tests conforming to internationally agreed test guidelines. There were studies in rats, mice and rabbits. No information is available on toxicity following repeated exposure to butanone oxime in humans.

Dose-related effects were observed in rats and mice in studies carried out by oral and inhalation routes. They were also noted in the developmental toxicity studies in rats and rabbits and in a two-generation toxicity study in rats.

Target organs of toxicity in experimental animals following repeated oral administration of butanone oxime, are the haematopoietic system, the nervous system, the liver and the urinary bladder. Effects on the nasal olfactory epithelium were also seen after exposure by the oral and inhalation routes.

The haemotoxic effects caused by butanone oxime are considered a borderline case for classification. There is no doubt that butanone oxime causes significant health effects following repeated exposure but the effects seen at dose levels approximately equal to the STOT RE 2 cut-offs are not considered as significant toxic effects according to the CLP guidance on haemotoxicity.

Conclusion from the DS before public consultation

There is no concern for specific target organ toxicity arising from a repeated oral or inhalation exposure to butanone oxime based on the available data. No classification for STOT RE is proposed.

Conclusion following public consultation

The anaemia and hepatic effects may both be considered as borderline significance for classification. Only the anaemic effect (reduction of haemoglobin > 10 %) in combination with the clinical signs of systemic toxicity in male rats at 100 mg/kg bw/day in the two-generation study strictly support classification in accordance with the guidance. As information was missing on the severity of related toxic effects (relative to the effects on the blood) and on the severity of the direct effects on the liver, the Dossier Submitter was unsure whether classification of butanone oxime with STOT RE 2 could be supported.

Comments received during public consultation

This hazard class originally wasn't opened for commenting; however two comments were received from Member States on this hazard class. In both cases, more details were requested on the severity of the findings in the blood and liver as well as careful consideration of these against the classification criteria.

Following the RAC meeting a targeted public consultation was launched for this hazard class. Two comments were provided by Industry in support of no classification. One Company based their comment on a lack of evidence of human health effects during use and the other believed that the effects observed in animals studies did not meet the criteria for classification. One Member state wrote in support of classification with STOT RE 2 due to adverse effects to the blood system. This MS commented that whilst the findings did not strictly meet the criteria on their own, the weight of evidence suggested severe damage to the blood system.

Assessment and comparison with the classification criteria

There are no relevant human data available with respect to repeated dose toxicity (i.e. no case reports, no epidemiological studies or medical surveillance reports). However, the specific target organ toxicity of butanone oxime following repeated oral and inhalation exposure of butanone oxime has been well tested in rats and mice. There were no studies carried out via the dermal route. The main adverse effects observed in these studies were to the blood, hepatic and respiratory systems. There were also some observations on the dams in a rabbit developmental toxicity study that will be considered below in the context of this endpoint.

Oral studies

There are five repeated dose studies available in rats and one in mice. Also available is a developmental study in rats and one in rabbits. All of these studies were carried out to guidelines similar or equivalent to OECD and all were carried out according to GLP, with the exception of one 90-day study in rats.

The results of the following oral studies in rats show that butanone oxime has an adverse effect on the blood system (in accordance with the Guidance on the Application of the CLP criteria for classification of substances inducing haemolytic anaemia) and contribute to the weight of evidence supporting classification.

Rats, 28-day (one study*)*

Sprague-Dawley rats (7/sex/dose) received 0, 4, 20 or 100 mg/kg bw/day butanone oxime by gavage for 28 days, with a 14 day recovery period. At ≥ 20 mg/kg, an increase in "reticulocyte ratio" was observed in males and females. In females only, an increase in platelet count and a decrease in RBC count, haematocrit and haemoglobin were observed. No further details were given. At 100 mg/kg, further effects in both males and females were noted. These included an increase in absolute and relative spleen weight, hypertrophy and hemosiderin granules. In the liver there was an increase in hemosiderin granules in Kupffer cells and extramedullary haematopoiesis was noted in both the liver and the spleen.

In the kidney there was evidence of lipofuscin-like substance in tubular epithelium. Most of these changes were reversible by the end of the recovery period.

This study indicates that the blood system is a target of butanone oxime. However, little information was given regarding incidence or severity of these findings, and most of the findings were reversible.

Rats, 90-day (3 studies)

In a well conducted study published in 1999, male and female F344 rats (10/sex/dose group) were exposed to butanone oxime in their drinking water for 13 weeks at doses of 0, 312, 625, 1 250, 2 500 or 5 000 ppm (equivalent to 0, 25/30, 50/65, 100/120, 175/215 and 280/335 mg/kg bw/day in males/females).

At doses relevant for classification (≤ 100 mg/kg bw/day), the only potentially relevant effects were seen on the blood system. Changes to blood parameters were noted in both males and females, although information is limited to the following.

There was a decrease in red blood cell count (up to 10 % when comparing to controls) in males when dosing at 100 mg/kg bw/day and above and in females (up to 6 % when comparing to controls) when dosed at 65 mg/kg bw/day and above. Reticulocyte counts were raised in males by up to 78 % higher than in controls at 100 mg/kg bw/day and above and in females, up to 25 % higher than controls from doses of 30 mg/kg bw/day and above. Haemoglobin levels were decreased in males, by a maximum of 5 % at doses of 100 mg/kg bw/day and above and in females, by a maximum of 2 % from doses of 65 mg/kg bw/day and above. There was also an increase in incidence and severity of haematopoietic cell proliferation in the spleen and bone marrow at doses ≥ 50/65 mg/kg bw/day in males/females. The figures available were maximum values and percentage changes at each dose level were not provided.

Blood toxicity was evident in both males and females at doses below and above the classification guidance values. The most significant effects were decreased erythrocyte count, increased reticulocyte count and increased methaemoglobin levels at 175/215 and 280/335 mg/kg bw/day. At the higher doses, signs of toxicity to the liver included Kupffer cell erythrophagocytosis and hemosiderin pigmentation. There was also an increase in tubular hemosiderin pigmentation in the kidney and degeneration of the nasal olfactory epithelium was noted in both sexes (posterior nasal section).

In a study published in 1993, Sprague-Dawley rats (10/sex/dose) were administered butanone oxime at 0, 40, 125 or 400 mg/kg bw by gavage (5 days/week). The only dose relevant for classification was 40 mg/kg bw/day. At this dose, effects indicating mild anaemia were observed. These included a decrease in red blood cell count (stated to be 16 % in males and 19.5 % in females) and a decrease in the haematocrit (5 % in males and 9.5 % in females). Increases in methaemoglobin (200 % in males and 140 % in females), leukocytosis (58 % in males and 49 % in females), compensatory reticulocytosis (325 % in males and 500 % in females) and Heinz body formation were all observed. Spleen weight was increased in both males and females (absolute, by 100 % in males and 60 % in females; relative, by 64 % in males and 75 % in females). Again, these effects were not considered sufficient for classification. Increases in methaemoglobin in this study,

following administration of butanone oxime, are indicative of a reduction in functional haemoglobin.

No details on effects observed at doses > 40 mg/kg bw/day were given. There were no details on effects on the liver.

In an unpublished study conducted in the 1970s, Sprague-Dawley rats (10/sex/dose) were administered 0, 25, 75 or 225 mg/kg bw/day butanone oxime (5 days/week). Only the low and mid doses were relevant for classification. At these doses changes in blood parameters indicative of haemolytic anaemia and compensatory haematopoiesis in the spleen and liver were noted in both males and females. However, no further details were given to inform on the magnitude or severity of these changes.

Rats, reproductive toxicity test (1 study)

In a 2-generation test, Sprague-Dawley rats (30/sex/dose) were exposed to 0, 10, 100 or 200 mg/kg bw/day butanone oxime for 5 days/week for 10 weeks during pre-mating and 3 weeks during the mating period. Effects in this study at doses 10 and 100 mg/kg bw/day are relevant for classification.

At the top dose of 200 mg/kg bw/day (above the limits for classification) there were a number of deaths.

F0 males: 4/30 deaths – 3/10 during pre-mating
F0 females: 11/30 deaths – further details not provided
F1 males: 15/30 deaths – 8/30 during pre-mating
F1 females: 8/30 deaths – 4/40 during pre-mating.

In this dose group, signs of anaemia were apparent and there was a disturbance of general behaviour, e.g. tremors, salivation, slow respiration, cyanosis, ataxia and convulsions.

At 10 mg/kg bw/day, red blood cell counts and haemoglobin were decreased in F0 males relative to controls (10 % and 6 % respectively). At necropsy F0 males were found to have darkened spleens (5/30 males) and spleen and liver extramedullary haematopoiesis and haemosiderosis were noted in males and females of both generations.

At 100 mg/kg bw/day, there was a consistent picture of anaemia in both males and females of both generations. Red blood cell count was decreased by 26/31 % in F0/F1 males and by 16/25 % in F0/F1 females and haemoglobin was also decreased by 9/14 % in F0/F1 males and by 8/11 % in F0/F1 females. In F0 and F1 males, methaemoglobin was increased by 82 % when compared to controls.

Clinical signs observed at 100 mg/kg bw/day were observed mainly in F0 males and females. These included lethargy, staggering, weaving and tremors.

At necropsy, both the absolute and relative spleen weight was increased (it is not clear which animal groups this was in) and signs of congestion were noted. Extramedullary haematopoiesis and haemosiderosis of the spleen and liver were observed in males and females of both generations. Details on the severity of these effects were not provided.

The findings of reduced haemoglobin and increased methaemoglobin at 100 mg/kg bw/day indicate a reduction in functional haemoglobin. This toxicity, coupled with increases of haemeosiderosis in the spleen and liver of males and females (also at 100 mg/kg bw/day) indicates a clear adverse effect on the blood system. As a similar toxic effect was not seen at the lower dose of 10 mg/kg bw/day, and the dose of 100 mg/kg bw/day in this study represents the cut off limit for classification these results alone are insufficient to support STOT RE 2. However, they contribute to the weight of evidence assessment.

Developmental Toxicity Study in rats

Further information is available from a developmental toxicity study in rats. Pregnant Sprague-Dawley rats (6/dose in a preliminary study and 25/dose in the main study) were given doses of 0, 25, 100, 200 or 400 mg/kg bw/day (preliminary study) and 0, 60, 200 or 600 mg/kg bw/day (main study) butanone oxime by gavage. The exposure duration was from GD 6-15. According to the guidance, for studies of duration 9 days or less, the guidance values used should be 10 % of the 90 day default guidance values. Therefore effects at doses of ≤ 1 000 mg/kg are considered for classification and all doses in this study are relevant for classification.

In the preliminary study, effects to the blood were noted from the lowest dose of 25 mg/kg bw/day. These included an increase in methaemoglobin on GD 16/20 (6/4 %) and an increase in reticulocyte count on GD 16/20 (18/14 %). At doses ≥ 100 mg/kg bw/day necropsy revealed enlarged spleens. At the top dose of 400 mg/kg bw/day animals were noted to have decreased body weight and also wobbly gait and general decreased responsiveness. The latter two effects were described as transient and are considered by RAC as acute symptoms of narcosis. Reticulocyte counts at this dose level, on GD 16/20 were 81/36 % and methaemoglobin at GD 16/20 was 39/9 %.

In the main study, enlarged spleens were noted in all treated animals but not in controls. Clinical signs of toxicity were seen at 200 and 600 mg/kg. These clinical signs were signs of general nervous system depression (wobbly gait, decreased responsiveness and urine stains) and were transient and had disappeared before dosing on the next day. No effects on the blood, spleen or liver were noted.

The effects observed in this study do not meet the criteria for classification but do provide further evidence that butanone oxime has an effect on the blood system. There is no indication that pregnant rats are more sensitive to the effects of butanone oxime than non-pregnant rats.

Mice, 90 days (1 study)

B6C3F1 mice (10/sex/dose) were administered butanone oxime in their drinking water at concentrations of 0, 625, 1 250, 2 500, 5 000 or 10 000 ppm (equivalent to 0, 110/145, 200/340, 515/630, 755/1 010 and 1 330/3 170 mg/kg bw/day). All doses used in this study were above the guidance limits for classification (STOT RE 2 ≤ 100 mg/kg bw/day). At the top two doses, there were effects indicating anaemia, effects on the urinary bladder and also degeneration of the nasal olfactory epithelium (described as minimal to moderate).

Developmental Toxicity Study in rabbits (1 study)

New Zealand White rabbits (5 females/dose in the preliminary study and 18 females/dose in the main study) were administered 0, 10, 20, 40 or 80 mg/kg bw/day butanone oxime by gavage in the preliminary study and 0, 8, 14, 24 and 40 mg/kg bw/day in the main study. The exposure duration was from GD 6-18 (12 days). Doses ≤ 600 mg/kg bw/day were relevant for classification with STOT RE 2 and doses ≤ 60 mg/kg bw/day were relevant for classification with STOT RE 1.

High levels of mortality were observed in both the preliminary and main study (see Acute Toxicity section for more details). At 40 mg/kg bw 2/5 rabbits died on GD 10-11 (after 4-5 doses) in the preliminary study and 8/18 died on GD 11-24 (after ≥ 5 doses) in the main study. Animals were described as having red/green urine, pale eyes and ears, pale liver and brown discoloured lungs. These deaths occurring after more than 3 doses may have been due to anaemia.

Increased reticulocyte counts and methaemoglobin were seen at gestation day 13 in the groups receiving 40 mg/kg butanone oxime in the main study, which was after 7 days dosing. It's not clear if these were signs of an acute effect, or of repeated dose toxicity. However, these parameters had essentially recovered by day 29. Increases in the reticulocyte count: at 10 mg/kg bw/day, GD 13/29: 9/5 %, and at 40 mg/kg bw/day on GD 13/29: 78/5 %. Increase in methaemoglobin: at 10 mg/kg on GD 13/29: 6/4 %, and at 40 mg/kg bw/day GD 13/29 42/9 %). According to CLP, premature deaths occurring after the first 3 days of treatment and signs of hypoxia, including cyanosis or pallor in anaemic animals meet the criteria for classification with STOT RE. In this study the anaemic status of the animals that died is a little uncertain, but the findings overall further contribute to the weight of evidence for classification.

Summary of significant adverse effects at doses relevant for classification following *oral* dosing:

Study (Ref)	Doses	Significant toxicity at doses relevant for STOT RE 1	Significant toxicity at doses relevant for STOT RE 2
Rats			
(TL12) 28-day, gavage, Sprague-Dawley rats (7/sex/dose) Equiv. OECD 407 GLP	0, 4, 20 and 100 mg/kg bw/day	*≤ 30 mg/kg bw/day*	*≤ 300 mg/kg bw/day* There were various changes indicative of anaemia (blood, spleen and liver) but the relevance to classification is unclear in the absence of information about incidence and severity.
(US NTP) 90-day, drinking water F344 rats (10/sex/dose)	0, 25/30, 50/65, 100/120, 175/215 and 280/335 mg/kg bw/day (males/females)	*≤ 10 mg/kg bw/day* All doses > 10 mg/kg bw/day	*≤ 100 mg/kg bw/day* <u>≥30 mg/kg bw/day:</u> Various changes indicative of anaemia (blood and spleen) but the relevance to classification is

Equiv. OECD 408 GLP			unclear in the absence of information about incidence and severity.
(TL9) 90-day, gavage, Sprague-Dawley rats (10/sex/dose) OECD 408 GLP	0, 40, 125 and 400 mg/kg bw/day (5 days/week)	*≤ 10 mg/kg bw/day* All doses > 10 mg/kg bw/day	*≤ 100 mg/kg bw/day* 40 mg/kg bw/day: ↓ RBC count (16/19.5 %) M/F ↓ Haematocrit (5/9.5 %) M/F ↑ Methaemoglobin (200/140 %) M/F ↑ Leukocyte counts: (58/49 %) M/F ↑ Reticulocyte counts: (325/500 %) M/F Further erthyocytic morphological changes (not specified) ↑ Spleen weight (rel. 100/60 % and abs. 64/75 % M/F)
(TL1) 90-day, gavage Sprague-Dawley rats (10/sex/dose) OECD 408 Non-GLP	0, 25, 75 and 225 mg/kg bw/day (5 days/week)	*≤ 10 mg/kg bw/day* All doses > 10 mg/kg bw/day	*≤ 100 mg/kg bw/day* <u>25 and 75 mg/kg bw/day:</u> There were various changes indicative of anaemia (blood and spleen) but the relevance to classification is unclear in the absence of information about incidence and severity.
(TL17) Two-generation, gavage, Sprague-Dawley rats (30/sex/dose; at least 20 pregnant females/group) Equiv. OECD 416 GLP	0, 10, 100 and 200 mg/kg bw/day Exposure duration: 10/11 week premating treatment, dosing during 3 weeks of mating, and in females continued dosing duration gestation and lactation.	*≤ 10 mg/kg bw/day* <u>10 mg/kg bw/day:</u> No significant toxicity at this dose.	*≤ 100 mg/kg bw/day* <u>100 mg/kg bw/day:</u> ↓ RBC count (26/31/16/25 %) M0/M1/F0/F1 ↓ Haemoglobin (9/14/8/11 %) M0/M1/F0/F1 ↑ Methaemoglobin in F0 and F1 males (82 %) ↑ Spleen weight (rel. and abs.) Extramedullary haematopoiesis and haemosiderosis in spleen and liver (F0 and F1 males and females)
(TL19) Developmental toxicity, gavage, Sprague-Dawley rats (females, 6/dose in preliminary study and 25/dose in main study) OECD 414 GLP	Preliminary study: 0, 25, 100, 200, 400 mg/kg bw/day Main study: 0, 60, 200, 600 mg/kg bw/day Exposure duration: 9 days	*≤ 100 mg/kg bw* <u>Preliminary study:</u> <u>≥ 25 mg/kg bw/day:</u> No significant toxicity at this dose. <u>Main study:</u>	*≤ 1000 mg/kg bw* <u>Preliminary study:</u> <u>≥ 100 mg/kg bw/day:</u> No significant toxicity at this dose. <u>400 mg/kg bw/day:</u> ↑ Methaemoglobin (GD 16/20): 39/9 % ↑ Reticulocyte count (GD 16/20): 81/36 %

		≥ 60 mg/kg bw/day: No significant toxicity at this dose.	Main study: ≥ 200 mg/kg bw/day: No significant toxicity at these doses.
Mice			
(US NTP) 90-day, drinking water B6C3F1 mice (10/sex/dose) Equiv. OECD 408 GLP	0, 110/145, 200/340, 515/630, 755/1 010, 1 330/3 170 mg/kg bw/day	*≤ 10 mg/kg bw/day* No doses relevant for classification with STOT-RE 1	*≤ 100 mg/kg bw/day* No doses relevant for classification with STOT RE 2
Rabbits			
(TL19) Developmental toxicity, gavage New Zealand White rabbits (Pregnant females: Preliminary study: 5/dose; main study 18/dose) OECD 414 GLP	Preliminary study: 0, 10, 20, 40 and 80 mg/kg bw/day Main study: 0, 8, 14, 24 and 40 mg/kg bw/day Exposure duration: 12 days (GD 6-18)	*≤ 60 mg/kg bw/day* Preliminary study: 10 mg/kg bw/day: No significant toxicity at this dose. 40 mg/kg bw/day: Mortality: 2/5 (GD 10 -11) Blood values: ↑ Reticulocytes ↑ Methaemo-globin (both reversible) Main study: 40 mg/kg bw/day: Mortality:8/18 (GD 11-24) Red/green urine, pale eyes and ears, pale liver, brown dicoloured lungs.	*≤ 600 mg/kg bw/day* Preliminary study: 80 mg/kg bw/day: Mortality (acute effect): 2/5 (≤ 48 h) and by GD 10, 5/5 females died. Dark red or red/green coloured urine, enlarged spleen, brown discoloured lungs. Main study: There were no doses within the guidance values for STOT RE 2.

Following oral administration of butanone oxime to rats, changes to the blood parameters, spleen and liver were observed that were indicative of anaemia. With the exception of a 90-day gavage study and a 2-generation study in rats, little information was given with regard to the incidence and severity of these findings. In these studies, changes to blood parameters were observed in both males and females. Spleen and liver weights were

increased and extramedullary haematopoiesis and haemosiderosis were reported in these organs (incidence and severity not given). Large increases in methaemaglobin were observed in both studies. Taking into account also the weight of evidence available from the other studies in rats, these observations of damage to the blood system indicate that classification for repeated dose toxicity may be necessary (see the section "Conclusions" below). In mice, no significant adverse findings were observed at doses relevant for classification following oral dosing. In a developmental study in rabbits, mortality occurred after 4 or more doses and changes to blood parameters were observed from a dose of 40 mg/kg bw/day. Animals were also described as having pale eyes and ears – potential indicators of hypoxia.

Inhalation studies

Studies to address inhalation toxicity after repeated exposure to butanone oxime were carried out in rats (28-day; 26-month) and mice (28-day; 18-month). All studies followed guidelines similar to or equivalent to OECD. Additionally, in mice, a 90-day study designed to specifically address effects observed to the nasal olfactory epithelium is available. All studies involved whole body exposure.

Rats, 28-days

F344 rats (numbers unknown/sex/dose) were exposed to vapours of 0, 30, 101 or 340 ppm (equivalent to 0, 0.22, 0.36 and 1.44 mg/L) butanone oxime. Changes to several blood parameters were observed, but only at the highest exposure level. This was relevant for classification with STOT RE 2 ($0.6 < C \leq 3$ mg/L). Decreases in haemoglobin, haematocrit, red blood cell count, mean corpuscular haemoglobin concentration (each parameter was found to be lowered by 10 % when compared to controls) were observed in both males and females. Increases in reticulocytes (3-fold), platelets (30 %) and leukocytes (13 %) were also observed in males and females. Spleen and liver weights were increased by 30 % in both sexes with no histopathological correlates.

The findings in this study do not fulfil the classification criteria for substances inducing haemolytical anaemia.

Rats, 26 months

In a combined toxicity and carcinogenicity study, F344 rats (80/sex/dose in the main carcinogenicity study and 10/sex/dose sacrificed at intervals of 3, 12 and 18 months) were exposed to butanone oxime at vapour concentrations of 0, 15, 75 or 374 ppm (equivalent to 0, 0.054, 0.270 and 1.346 mg/L).

At 3 months, adverse effects were seen in the blood and to the spleen, including small increases or decreases in blood parameters and increased spleen weight but these were limited to the males and females in the highest exposure group. In males of the top exposure group only, congestion of the spleen was noted and hemosiderin pigmentation and extramedullary haematopoiesis. These findings were at doses above the guidance limit for classification.

At 12 and 18 months, there were increased incidences of congestion of the spleen in males and females at 0.054 mg/L. This exposure level was within the guidance limits for classification.

At the higher exposure levels, exceeding the guidance limits for classification, there were also effects indicative of anaemia. These changes were found to be reversible, by 18 months in males and by 26 months in females.

At the end of the study (26 months), the only exposure level relevant for classification was 0.054 mg/L. No splenic congestion was noted in either males or females. In males, an increase in spongiosis hepatis was observed. According to the scientific literature, this is a distinct lesion that may be associated with certain forms of hepatic neoplasia. A dose-related increase in incidence and severity of degeneration of the nasal olfactory epithelium in the dorsal meatus of males and females was also evident. No further details were provided.

The non-neoplastic findings in this study did not fulfil the criteria for classification.

Mice, 28-days

CD-1 mice were exposed to 0, 30, 101 or 341 ppm butanone oxime vapour (0, 0.11, 0.36 and 1.44 mg/L). All these levels were relevant for classification (≤ 3 mg/L STOT RE 2, ≤ 0.6 mg/L STOT RE 1). The only significant finding was at the top exposure level of 1.44 mg/L, where spleen weight was increased by 30 % in both males and females. No histopathology was available. There were no effects reported in this study to warrant classification.

Mice, 18-months

A combined repeated dose toxicity and carcinogenicity study is available in CD-1 mice. Males and females (60/sex/dose in the main carcinogenicity study and 10/sex/dose at intervals of 3 and 12 months) were exposed to whole body vapours of 0, 15, 75 or 374 ppm butanone oxime (equivalent to 0, 0.054, 0.270 and 1.346 mg/L).

At 3 months, there were no treatment-related effects observed.

At 12 and 18 months, effects to the liver and respiratory system were observed at the only exposure level relevant for classification (0.054 mg/L). In the liver there was increased hemosiderin in reticuloendothelial cells and an increase in centrilobular hypertrophy in both males and females. Granulomatous inflammation was observed in males (43 % affected versus 24 % in controls) and females (43 % affected versus 32 % in controls) and there was a slight increase in incidence of necrosis (females only). Degenerative changes and formation of replacement tissue in the olfactory epithelium in the nasal turbines was noted in both males and females. No further details were provided.

At exposure levels above the guidance limits for classification, small changes to blood parameters, indicative of anaemia were noted. The effects described at the only exposure level relevant for classification do not fulfil the criteria for classification with STOT RE.

The same laboratory followed this study up with a specific investigation of the potential of butanone oxime to damage the olfactory epithelium of mice during a 90-day inhalation period. It was found that exposure levels as low as 0.108 mg/L resulted in damage to this tissue (see table below). As discussed in the section on Specific Target Organ Toxicity following a single exposure, these effects are considered to be a result of short-term or acute exposure and do not increase in severity on repeated exposure. Therefore they are not considered further in the context of classification for repeated dose effects.

Summary of adverse effects occurring at exposure concentrations relevant for classification following *inhalation* exposure:

Study (Ref)	Exposure	Significant toxicity at exposures relevant for STOT RE 1	Significant toxicity at exposures relevant for STOT RE 2
Rats			
(TL4) 28-day F344 rats (unknown number/sex/dose) Equiv. OECD 412 GLP	0, 0.22, 0.36, 1.44 mg/L Whole body exposure to vapour 6 h/day, 5 days/week for 4 weeks	*≤ 0.6 mg/L* There were no effects at doses relevant for classification with STOT RE 1.	*≤ 3 mg/L* <u>1.44 mg/L:</u> ↓ Haemoglobin (10 %) ↓ Haematocrit (10 %) ↓ RBC count (10 %) ↓ MCHC (10 %) ↑ Reticulocytes (threefold) ↑ Platelets (30 %) ↑ Leucocytes (13 %) All in males and females Spleen and liver: ↑ Weights (30 %) in males and females
(TL18) Combined chronic toxicity and carcinogenicity study F344 rats (80/sex/dose in the main carcinogenicity study and 10/sex/dose sacrificed at intervals of 3, 12 and 18 months) Equiv. OECD 453 GLP	0, 0.054, 0.270 and 1.346 mg/L Whole-body exposure to vapour (MMAD: 2.1-2.7 µm, GSD: 2.7-3.4), 6 h/day, 5 days/week Exposure duration: 26 months	*At 3 months: (≤ 0.2 mg/L)* No effects *At 12/18/26 months: (≤ 0.05/0.03/0.02 mg/L)* All doses in the study were > than the guidance values.	*At 3 months: (≤ 1.0 mg/L*) No effects observed at the only level relevant for classification. *At 12 months (≤ 0.25 mg/L)* *At 18 months: (≤ 0.17 mg/L)* <u>0.054 and 0.054 mg/L:</u> ↑ Congestion in the spleen (males and females) *At 26 months: (≤ 0.11 mg/L)* <u>0.054 mg/L:</u> ↑ Spongiosis hepatis in the liver of males

			↑ Degeneration of the olfactory epithelium in the dorsal meatus (males and females)
Mice			
(TL4) 28 day CD-1 mice (unknown numbers/sex/dose) Equiv. OECD 412 GLP	0, 0.11, 0.36 and 1.44 mg/L Whole-body exposure Vapour	*≤ 0.6 mg/L* There were no effects at doses relevant for classification with STOT RE 1.	*≤ 3 mg/L* No significant adverse effects observed.
(TL14) 90 day, investigation of repeated exposure and damage to the olfactory epithelium CD-1 mice (main study: 10 males/dose, satellite groups 5 males/dose) Equiv. OECD 413 GLP	0, 0.011, 0.036, 0.108 and 0.360 mg/L Whole-body exposure Vapour Exposure duration: 6h/day, 5 days/week for 1, 2, 4 or 13 weeks Recovery period of 4 or 13 weeks	*≤ 0.2 mg/L* <u>≥ 0.036 mg/L:</u> Degeneration of the olfactory epithelium in dorsal meatus (10 % of the tissue affected) (dose-related increase in incidence and severity) – observed after 1,2, 4 and 13 weeks Recovery within 4 weeks <u>≥ 0.108 mg/L:</u> Replacement by squamous/squamoid respiratory epithelium – observed after 1 week Recovery within 13 weeks	*≤ 1 mg/L* <u>0.360 mg/L:</u> Degeneration of the olfactory epithelium in dorsal meatus (10 % of the tissue affected) (dose-related increase in incidence and severity) – observed after 1, 2, 4 and 13 weeks Recovery within 4 weeks Replacement by squamous/squamoid respiratory epithelium – observed after 1 week Recovery within13 weeks
Combined chronic toxicity and carcinogenicity CD-1 mice (60/sex/dose in the main carcinogenicity study and 10/sex/dose sacrificed at intervals of 3 and 12 months) Equiv. OECD 453 GLP	0, 0.054, 0.270 and 1.346 mg/L Whole-body exposure to vapour (MMAD: 2.1-2.7 µm, GSD: 2.7-3.4), 6 h/day, 5 days/week Exposure duration: 18 months	*At 3 months: (≤ 0.2 mg/L)* No effects *At 12 and 18 months: (≤ 0.05/0.03/0.02 mg/L)* All doses in the study were > than the guidance values.	*At 3 months: (≤ 1.0 mg/L)* No effects were observed at doses relevant for classification. *At 12 months (≤ 0.25 mg/L) and 18 months (≤ 0.17 mg/L)* <u>0.054 mg/L:</u> Liver: ↑ Hemosiderin in reticuloendothelial cells ↑ Centrilobular hypertrophy in males and females

			↑ Granulomatous inflammation in 43 % males and females (versus 24 % in control males and 32 % in control females) ↑ Necrosis in females (slight) Respiratory system: Degenerative changes and formation of replacement tissue on the olfactory epithelium in the nasal turbines (males and females)

Following repeated inhalation exposure of rats to butanone oxime, effects on the blood, spleen and liver were observed. After 28 days, signs of anaemia were present and liver and spleen weights were increased. In a 26-month study, congestion of the spleen was noted at 12 and 18 month intervals, but this was not seen at the end of the study. In mice, no adverse effects were reported at exposures relevant for classification following 28-day exposure to butanone oxime. In an 18-month study, effects on the liver were seen in males and females. These included an increase in hemosiderin in reticuloendothelial cells, centrilobular hypertrophy, and an increase in granulomatous inflammation. In females only, there was a slight increase in necrosis of the liver. The incidence and severity of these effects were not reported and therefore these data alone are insufficient to justify classification but contribute to the weight of evidence.

Dermal studies

No repeated dose studies were carried out via the dermal route.

Conclusions

Effects to the blood

There are several oral and inhalation studies reporting effects indicative of anaemia at doses sufficiently low to justify classification. These effects have been seen in rats and rabbits.

Although full descriptions of the severity of the effects seen on the blood system are not available, the consistent nature of the findings reported is a concern. The CLP guidance provides examples of effects fulfilling classification criteria for substances inducing anaemia. For butanone oxime, there are several aspects of its toxicity to the blood system that can be related to these criteria, supporting classification for STOT RE:

- Premature deaths in anaemic animals that are not limited to the first three days of treatment in the repeated dose study. (Mortality during days 0-3 may be relevant for acute toxicity.)
- Clinical signs of hypoxia, e.g. cyanosis, dyspnoea, pallor in anaemic animals that are not limited to the first three days of treatment in the repeated dose study.

- Reduction in functional Hb at ≥ 20 % due to a combination of Hb reduction and MetHb increase.
- Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %) in a 28-day study.
- Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis.

As detailed in the tables above, the doses at which these effects were observed were sufficiently low to match the criteria for a category 2 classification. It is concluded that butanone oxime should be classified as **STOT RE 2; H373 (Causes damage to the blood system through prolonged or repeated exposure)**.

Effects on the spleen and liver

Both the spleen and liver have been affected adversely in rats and mice following long-term, repeated exposure to butanone oxime. In rats, increases in spleen and liver weight were observed along with congestion of the spleen and extramedullary haematopoiesis and haemosiderosis of the spleen and liver following oral administration. In mice, effects on the liver were noted only in a lifetime inhalation study. In this study there was an increase in hemosiderin in reticuloendothelial cells, centrilobular hypertrophy, an increase in granulomatous inflammation and "slight" increase in the incidence of necrosis (in females only). There were no adverse effects on the spleen of mice in any study at doses relevant for classification.

From the limited information available, it appears that the toxicity observed at the level of concentrations relevant for classification was not marked. Therefore, no classification is considered appropriate. However, these effects on the spleen and liver provide further support of an anaemic effect of butanone oxime to rats and mice.

Effects on the olfactory epithelium

As presented above and in the section on STOT SE, effects on the nasal olfactory epithelium were observed in rats and in mice following repeated exposure. However, RAC is of the opinion that this toxicity arises as a consequence of repeated single or short term exposures and therefore it is not considered relevant for classification with STOT RE.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 22: Summary table of relevant *in vitro* mutagenicity studies

Method	Results	Remarks	Reference
In vitro study **Bacterial gene mutation test (Ames test,** gene mutation), equivalent or similar to OECD TG 471/EU B.14; GLP compliant S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Doses tested: 0, 0.1, 0.5, 1.0, 2.5, 5.0 mg/plate; solvent: ethanol (50 µL/plate) Control: neg/ pos.: yes	**Negative** No mutagenic effects For S. typhimurium TA1535, TA1537, TA1538, TA98, TA 100; in concentrations up to 5.0 mg/plate); met. act.: with and without Cytotoxicity: 2.5 and 5.0 mg/plate in all strains except TA98 in absence of S9 mix, rat; TA1537 in presence of S9 According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test according to the plate incorporation and pre-incubation method Test material: butanone oxime, purity: 99.5 %	TL2 (1983), unpublished study report, confidential
In vitro study **Mammalian cell gene mutation assay** (mouse lymphoma study; gene mutation), equivalent or similar to OECD TG 476/ EU B.17, GLP compliance not specified Mouse lymphoma L5178Y cells met. act.: with and without S9 activation Dose: up to 6.5 µL/mL L5178Y/TK$^{+/-}$ Mouse lymphoma assay Control: neg./pos.: yes	**Negative** negative with and without metabolic activation (with metabolic activation weak positive effects at both highest doses 5.5 and 6.5 µL/mL without relevance due to high induced cytotoxicity) Cytotoxicity: yes, at all doses tested (↑ depending on the dose) According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: 99.5 %	Rogers-Back et al. (1988)
In vitro study **DNA damage and repair, unscheduled DNA synthesis in mammalian cells in vitro** (DNA damage and/or repair), comparable to OECD TG 482/EU B.18 (Genetic toxicology: DNA damage and repair, unscheduled DNA synthesis in mammalian cells in vitro), GLP compliance not specified Hepatocytes: male F344 rats Dose: up to 5000 pg/mL Control: neg./pos.: yes	**Negative** negative for hepatocytes: male F344 rats (all strains/cell types tested); met. act.: not applicable; Cytotoxicity: yes (5000 and 1500 pg/mL) According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: confidential (> 98 %)	TL11 (1995), unpublished study report, confidential
In vitro study **In vitro sister chromatid exchange assay in mammalian cells,** Comparable to OECD TG 479/EU B.19 (Genetic toxicology: In vitro sister chromatid exchange assay in mammalian cells); GLP compliance assumed CHO cells Dose: up to 500 µg/mL (-S9); 5000 µg/mL (+S9); Control: neg./pos.: yes	**Negative** No induction of sister chromatid exchanges (SCE) in cultured Chinese hamster ovary (CHO) cells with and without S9 activation According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: 99.5 %	NTP (1999)

Method	Results	Remarks	Reference
In vitro study **In vitro mammalian chromosome aberration test, c**omparable to OECD TG 473/EU B.10, GLP compliance assumed CHO cells Dose: up to 5000 µg/mL (-/+S9) Control: neg./pos.: yes	**Negative** No induction of chromosome aberration in cultured CHO cells with and without S9 activation Up to 200 first-division metaphase cells were scored/dose According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: 99.5 %	NTP (1999)

Table 23: Summary table of relevant *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
In vivo study Sex-linked recessive lethal (SLRL) test in Drosophila melanogaster (gene mutation), comparable to OECD TG 477/EU B.20 (Genetic Toxicology: Sex-linked recessive lethal test in Drosophila melanogaster), GLP compliant Drosophila melanogaster, male (15/dose) **oral**: feed; neg./pos. control: yes Doses tested:7500 ppm in 5 % sucrose in aqueous solution (nominal in diet), Exposure duration: 3 consecutive days;	**Negative** No evidence of mutations in the post-meiotic germ cells of male Drosophila melanogaster toxicity: yes According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: 98.5 %	TL21 (1991), unpublished study report, confidential
In vivo study Chromosome aberration assay in Sprague-Dawley rats (chromosome aberration), comparable to OECD TG 475/EU B.11 (Mammalian bone marrow chromosome aberration test) **Rats**, Sprague-Dawley, male and female (5/dose); **oral** (gavage); control: neg./pos.: yes Doses tested: 300, 600, 1200 mg/kg bw (nominal in water); Exposure duration: single dose; observation period: 6, 24, 48h	**Negative** No significant increase in chromosome aberrations in the bone marrow of male/female rats toxicity: yes, clinical signs within 4 hours of dosing at each dose level tested According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: 99.98 %	TL11 (1990), unpublished study report, confidential
In vivo study Mouse peripheral blood micronucleus test in B6C3F1 mice (clastogenic/genotoxic potential), comparable to OECD TG 474/EU B.12 (Mammalian erythrocyte micronucleus test), GLP compliant **Mice**, B6C3F1, male/female (5/sex/group); **oral** (drinking water), control: neg: yes Doses tested: 0, 625, 1250, 2500, 5000 or 10,000 ppm (nominal in water) (m: up to 1330 mg/kg bw; f: up to 3170 mg/kg bw) Exposure duration: daily, 13 weeks	**Negative** No increase in the frequency of micronucleated normochromatic erythrocytes in the peripheral blood (males/females) 10000 ppm, m/f: ↓ (76/79.8 %) in proportion of normochromatic erythrocytes among the total erythrocyte population toxicity: yes According to CLP: **no classification for germ cell mutagenicity**	**Key study** Test material: butanone oxime, purity: 99.5 %	NTP (1999)

Method	Results	Remarks	Reference
In vivo study The potential to produce DNA and RNA adducts in the liver by butanone oxime, No guideline followed, GLP compliant **Rat**, Wistar, male/female **inhalation**, Positive control: 2-nitropropane Doses tested: 375 - 1000 ppm (1350 - 3600 mg/m^3) Exposure duration: 6h	**Negative** Rat liver: no DNA adduct formation Additional information: Induction of adducts in rat liver RNA Positive control: ↑ 8-oxodeoxyguanosine, N^2-aminodeoxyguanosine and 8-aminodeoxyguanosine in rat liver	**Key study** Test material: butanone oxime, purity: 99.5 %	TL10 (2000), unpublished study report, confidential; Friedewald et al. (2001); Völkel et al. (1999)

4.9.1 Non-human information

4.9.1.1 In vitro data

Butanone oxime was tested for germ cell mutagenicity in the following in vitro studies: bacterial reverse mutation assays (Ames test) conducted by several methods in standard bacterial strains in the presence or absence of rat liver activating enzymes (comparable to OECD TG 471/EU B.14), a further single bacterial reverse mutation assay conducted by the pre-incubation method with and without metabolic activation, a mouse lymphoma study (comparable to OECD TG 476/EU B.17), and an UDS test (comparable to OECD TG 482/EU B.18). In addition, in cytogenetic tests with cultured Chinese hamster ovary cells (CHO) the induction of sister chromatid exchanges (comparable to OECD TG 479/EU B.19) and the chromosome aberration (comparable to OECD TG 473/EU B.10) was evaluated both in the presence or absence of S9 activation. In all studies butanone oxime was used as test material. The results of in vitro studies on germ cell mutagenicity are summarised in Table 22 (s. there).

Butanone oxime did not induce reverse mutations in Salmonella typhimurium strains (TA 1535, TA 1537, TA 1538, TA 98, TA 100; in concentrations up to 10000 µg/plate) or Escherichia coli (WP2 uvr A) in the presence or absence of rat or hamster liver activating enzymes (TL2, 1983, unpublished study report, confidential; TL12, 1996, unpublished study report, confidential; Rogers-Back et al. 1988; NTP 1999). A single bacterial reverse mutation assay conducted by the pre-incubation method reported a mutagenic response in only tester strain TA 1535 and only in the presence of high (not standard) levels of hamster liver activating enzymes (NTP 1999). A mouse lymphoma study found evidence of mutagenic activity in mouse lymphoma L5178Y cells in the absence of S9 activation but in the presence of cytotoxicity (growth inhibition of 50-92.5 % at doses of 2.8-6.5 µL/mL). Following S9 activation, a negative response was observed (Rogers-Back et al. 1988). A DNA damage and repair assay, unscheduled DNA Synthesis (UDS) test in rat primary hepatocytes (male F344 rats; tested concentrations 5000 and 1500 pg/mL) was negative (TL11, 1995, unpublished study report, confidential). In cytogenetic tests with cultured CHO cells, no induction of SCE was observed at concentrations up to toxicity (500 µg/mL) in the absence of S9 or up to the assay limit (5000 µg/mL) in the presence of S9, in addition, no increase in chromosomal aberrations was observed in cultured CHO cells treated with up to 5000 µg/mL butanone oxime, with or without S9 (NTP 1999).

4.9.1.2 In vivo data

Butanone oxime was investigated in a Drosophila melanogaster sex-linked recessive lethal (SLRL) test (comparable to OECD TG 477/EU B.20), a chromosome aberration assay in Sprague-Dawley rats (comparable to OECD TG 475/EU B.11), for its clastogenic/genotoxic potential in vivo in a mouse micronucleus test in the peripheral blood (comparable to OECD TG 474/EU B.12), and for its potential to produce DNA and RNA adducts in the liver in a study with rats exposed by inhalation to 375-1000 ppm (1350-3600 mg/m^3) butanone oxime for 6 hours. In all studies butanone oxime was used as test material. The results of in vivo studies on germ cell mutagenicity are summarised in

Table 23 (s. there).

A Drosophila melanogaster SLRL test (for sex-linked recessive mutations) showed no evidence of mutations in the post-meiotic germ cells of male Drosophila melanogaster when administered 7500 ppm butanone oxime in their feed for three consecutive days (TL21, 1991, unpublished study report, confidential). In a chromosome aberration assay in male and female Sprague-Dawley rats no significant increase in chromosomal aberrations in the bone marrow was found after single oral doses by gavage of up to 1200 mg/kg bw butanone oxime (TL11, 1990, unpublished study report, confidential). In a mouse peripheral blood micronucleus test no increase in the frequency of micronucleated normochromatic erythrocytes was observed in the peripheral blood of male or female B6C3F1 mice administered up to 1330/3170 mg/kg bw/d butanone oxime via drinking water for 13 weeks. The percentage of normochromatic erythrocytes among the population of circulating erythrocytes was markedly decreased at the highest dose tested (1330/3170 mg/kg bw/d) in male and female mice (NTP 1999). The potential for the formation of DNA and RNA-adducts by butanone oxime was investigated in liver DNA and RNA from male and female rats exposed to butanone oxime by inhalation for 6 hours. DNA adducts could not be observed, additionally information is given by induction of DNA adducts in rat liver RNA (TL10, 2000, unpublished study report, confidential; Friedewald et al. 2001; Völkel et al. 1999).

4.9.2 Human information

No information is available.

4.9.3 Other relevant information

No information is available.

4.9.4 Summary and discussion of mutagenicity

The possibility that butanone oxime may induce heritable mutations in the germ cells of humans were examined in tests by in vitro and in vivo methods. No information is available in humans.

In vitro: Butanone oxime did not induce reverse mutations in Salmonella typhimurium strains or Escherichia coli. The tests were conducted up to the limit dose recommended by guideline and cytotoxicity was noted at the highest tested dose level. A single reverse mutation bacterial assay conducted by the pre-incubation method reported a mutagenic response in only tester strain TA 1535 and only in the presence of high (not standard) levels of hamster liver activating enzymes. In mammalian in vitro systems, butanone oxime did not induce chromosomal aberrations in rat hepatocytes, gene mutation in mouse lymphoma cells, sister chromatid exchange or chromosome aberrations in cultured CHO cells.

In vivo: Butanone oxime did not induce mutations in the post-meiotic germ cells of male Drosophila melanogaster and micronuclei in peripheral blood erythrocytes in male and female B6C3F1 mice treated via drinking water, and showed no significant increase in chromosomal aberrations in the bone marrow of rats. In liver DNA from butanone oxime exposed rats by inhalation for 6 hours, DNA adducts could not be observed.

4.9.5 Comparison with criteria

Hazard classification for germ cell mutagenicity primarily aims to identify substances causing heritable mutations or being suspected of causing heritable mutations. A secondary aim is that the mutagenicity in germ cell as well as in soma cells offers supporting information with respect to the mode of action of carcinogenic substances. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.

Hazard categories for germ cell mutagens:

'Category 1: Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.

Category 1A: The classification in Category 1A is based on positive evidence form human epidemiology studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.

Category 1B: The classification in Category 1B is based on positive result(s) from

- in vivo heritable germ cell mutagenicity tests in mammals; or
- in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to drive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.'

No data are available which justify a classification of butanone oxime as mutagen Category 1 in accordance with CLP.

'Category 2: Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on:

Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

- Somatic cell mutagenicity tests in vivo, in mammals; or
- Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.'

The evaluation of the genotoxic potential of butanone oxime based on a battery of in vitro and in vivo tests conforming to internationally agreed test guidelines. It is concluded that for butanone oxime no genotoxic potential in vivo could be established.

4.9.6 Conclusions on classification and labelling

There is no concern for direct genotoxic properties of butanone oxime based on the available data from in vitro and in vivo tests.

According to CLP classification of butanone oxime for germ cell mutagenicity is not warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Butanone oxime was tested in the following *in vitro* studies: bacterial reverse mutation assays (Ames test) conducted by several methods in standard bacterial strains in the presence or absence of rat or hamster liver activating enzymes (comparable to OECD TG 471), a further single bacterial reverse mutation assay conducted by the pre-incubation method with and without metabolic activation, a mouse lymphoma study (comparable to OECD TG 476), and a UDS test (comparable to OECD TG 482). In addition, in cytogenetic tests with cultured Chinese hamster ovary cells (CHO) the induction of chromosome aberrations (comparable to OECD TG 473) and sister chromatid exchanges (comparable to OECD TG 479) was evaluated both in the presence or absence of S9 activation.

The test substance did not induce reverse mutations in *Salmonella typhimurium* or *Escherichia coli*. The tests were conducted up to the limit dose recommended by the guideline, and cytotoxicity was noted at the highest tested dose level. A single reverse mutation bacterial assay conducted by the pre-incubation method reported a mutagenic response in tester strain TA 1535 only, in the presence of high (non-standard) levels of hamster liver activating enzymes.

Butanone oxime was also tested *in vivo*, in a chromosome aberration assay in Sprague-Dawley rats (comparable to OECD TG 475), in a mouse peripheral blood micronucleus test (comparable to OECD TG 474), and for its potential to produce DNA and RNA adducts in the liver in a study with rats exposed by inhalation to 375-1 000 ppm (1 350-3 600 mg/m^3) butanone oxime for 6 hours.

Clear negative results were seen in both the chromosome aberration assay and the micronucleus test. Butanone oxime did not induce micronuclei in peripheral blood erythrocytes in male and female B6C3F1 mice treated via drinking water, and showed no significant increase in chromosomal aberrations in the bone marrow of rats. In liver DNA from rats exposed to butanone oxime by inhalation for 6 hours, DNA adducts were not observed.

There is no concern for the germ cell mutagenicity of butanone oxime based on the available data from *in vitro* and *in vivo* tests. The DS concluded that according to CLP, classification of butanone oxime for germ cell mutagenicity is not warranted.

Comments received during public consultation

One MS stated their support for no classification for this endpoint. In commenting on the absence of genotoxicity as a potential mode of action for butanone oxime carcinogenicity, the expert comments received from industry and academia indirectly supported this view.

Assessment and comparison with the classification criteria

Butanone oxime has been tested for mutagenicity in a number of *in vitro* and *in vivo* studies.

In vitro

In a bacterial mutagenicity assay, butanone oxime was tested at 0, 0.1, 0.5, 2.5 and 5.0 mg/plate against *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 in both the presence and absence of rat liver S9. Cytotoxicity was observed at the top two concentrations in all strains, except for TA98 (-S9) and TA1537 (+S9). No mutagenic response was observed in any strain; adequate positive and negative controls were included.

Although the Dossier Submitter provided very limited details, it is apparent that a negative result was also seen in a further bacterial mutagenicity assay employing both *S. typhimurium* and *E. coli* tester strains. In this study, both rat liver and hamster liver S9 was used to provide exogenous metabolic activation. A further test, in *S. typhimurium* TA1535 only, was reported to give a positive result. However, this was only in the presence of a high and non-standard concentration of hamster liver S9.

Overall, when tested according to regulatory protocols, butanone oxime has repeatedly given negative results in bacterial assays. The relevance of the single positive result is limited due to the non-reproducibility of the finding and the unusual test protocol used.

A mammalian cell gene mutation assay was carried out using mouse lymphoma L5178Y cells. Butanone oxime was incubated with the cultured cells at concentrations of 0, 2.8, 3.6, 4.6, 5.5 and 6.5 µL/mL (-S9) and 0, 1.7, 2.8, 3.6, 4.6, 5.5 and 6.5 µL/mL (+S9). Positive and negative controls behaved accordingly. No mutagenic activity was observed in the presence of S9. In contrast, in the absence of S9, there was an increase in the mutant frequency (see following table). However, this was small in magnitude and was only seen

at the top 2 concentrations, at which high levels of cytotoxicity were observed (12.5 and 7.5 % relative total growth at these 2 concentrations).

In the absence of S9:

Concentration (µL/mL)	Relative Total Growth (% of control)	Mutant Frequency (/100 000 survivors)
0	100	0.50
2.8	50	0.75
3.6	35.5	0.95
4.6	28.5	1.30
5.5	12.5	2.00
6.5	7.5	2.65

As recognised in the mouse lymphoma test guideline, excessive levels of cytotoxicity can lead to false positive results. The reductions in relative total growth at the top two concentrations in this assay are indicative of excessive toxicity and therefore, the overall result of this study is negative for mutagenicity.

In a chromosome aberration test, Chinese hamster ovary (CHO) cells were treated with butanone oxime up to 5 000 µg/mL in the presence and absence of rat liver S9. Adequate positive and negative controls were included. No induction of chromosome aberration was observed with or without S9. This study was negative for mutagenicity.

A sister chromatid exchange (SCE) assay was also conducted. CHO cells were treated with butanone oxime up to 5 000 µg/mL and 500 µg/mL in the presence and absence of rat liver S9, respectively. Adequate positive and negative controls were included. The results showed no induction of SCEs with or without activation. No further study details were given. This study was negative for germ cell mutagenicity.

In an unscheduled DNA synthesis in mammalian cells, hepatocytes from male F344 rats were incubated with 0, 15, 50, 150, 500, 1 500 or 5 000 pg/mL butanone oxime. An appropriate positive control was included and behaved accordingly. Cytotoxicity was observed at the top two doses. The results of this study showed no increase in the number of net nuclear grain counts at any dose level. The result of this study is negative for damage to DNA synthesis in rat hepatocytes.

In vivo

In a chromosome aberration test, Sprague Dawley rats (5/sex/dose) were given a single oral dose of butanone oxime by gavage. The doses employed were of 0, 300, 600 or 1 200 mg/kg bw. An appropriate positive control was included. Bone marrow cells, arrested in metaphase and collected at 6, 24 and 48 hours following administration were examined for structural chromosome aberrations.

Clinical signs of toxicity (not specified) were observed within 4 hours of dosing. The results of this study showed no significant increases in percentages of aberrant cells in treated groups at any time point. The positive control behaved accordingly.

In an *in vivo* micronucleus test, B6C3F1 mice (5/sex/dose) were administered butanone oxime in their drinking water at doses of 0, 110/145, 200/340, 515/630, 755/1 010 or 1 330/3 170 mg/kg bw/day for 13 weeks. Blood samples were obtained at the end of the study and evaluated for the frequency of micronucleated cells among normochromatic erythrocytes.

At the highest dose tested, the population of circulating erythrocytes was markedly decreased (76 % decrease in males and 79.8 % decrease in females). There was no increase in the frequency of micronucleated normochromatic erythrocytes observed in male or female mice at any exposure concentration.

The results of these two tests are consistent with the findings seen *in vitro* and show that butanone oxime lacks the potential to damage chromosomes.

In a further study, a single inhalation exposure of 6 hours to concentrations of 1.35-3.6 mg/L did not lead to the formation 8-amino-deoxyguanosine or 8-oxo-deoxyguanosine adducts in the livers of male and female Wistar rats. In contrast, such adducts were found following treatment with the genotoxic carcinogen, 2-nitropropane.

Classification

The mutagenic potential of butanone oxime has been studied in a series of standard and modified *in vitro* and *in vivo* tests. Apart from isolated observations of apparent treatment-related increases in mutagenic frequency induced under extreme conditions in one *S. typhimurium* tester strain and in a mouse lymphoma assay, the studies consistently show butanone oxime to be non-genotoxic. There are no positive *in vivo* studies.

As butanone oxime has been shown to be non-genotoxic, RAC is in agreement with the Dosser Submitter that **no classification for germ cell mutagenicity is warranted**.

4.10 Carcinogenicity

Table 24 Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Chronic toxicity and carcinogenicity study, similar to OECD TG 453/EU B.33, GLP compliant **Rat**; F344; male/female (80/sex/dose; 10/sex/dose/ interval) **Inhalation:** vapour (particle size distribution: MMAD: 2.3-2.6 µm, GSD: 2.1-2.8), whole body Doses tested: Exposure duration: 6h/d, 5d/wk for 26 months, interim sacrifice at 3, 12 and 18 months	**Positive: Malignant and benign liver tumours** **Carcinomas in male rats exposed by inhalation to 374 ppm (1346 mg/m³), and adenomas, dose-related increase in males at 15 ppm (54 mg/m³) and higher** 0, 15, 75, 374 ppm, equivalent to 54, 270, 1346 mg/m³, **males: liver carcinomas** 0/50, 0/51, 1/51, 12/51; statistically significant at 374 ppm 0, 15, 75, 374 ppm, equivalent to 54, 270, 1346 mg/m³, **males: liver adenomas** 0/50, 2/51, 5/51, 18/51; statistically significant at 75 and 374 ppm 0, 15, 75, 374 ppm, equivalent to 54, 270, 1346 mg/m³, **males: fibroadenomas in mammary gland** (2/50, 2/50, 4/50, 9/50; statistically significant at 374 ppm 0, 15, 75, 374 ppm, equivalent to 54, 270, 1346 mg/m³, females: liver adenomas 0/50, 0/50, 2/50, 4/51; not statistically significant 0, 15, 75, 374 ppm, equivalent to 54, 270, 1346 mg/m³, females: fibroadenomas in mammary gland 10/50, 7/50, 9/50, 17/50; not statistically significant **$LOAEC_{sys, m}$ = 15 ppm (54 mg/m³) for liver tumour development** **$NOAEC_{sys}$ not available** According to CLP butanone oxime fulfils the criteria for classification as **Carc. 1B, H350: May cause cancer**	**Key study** Test material: butanone oxime, purity: > 99.9 %	Newton et al. (2001); TL18 (1994), unpublished study report, confidential

Chronic toxicity and carcinogenicity study, similar to OECD TG 453/EU B.33, GLP compliant **Mouse**; CD-1; male/female (60/sex/dose; 10/sex/dose/ interval) **Inhalation:** vapour (particle size distribution: MMAD: 2.1-2.7 µm, GSD: 2.7-3.4), whole body Doses tested: 0, 15, 75, 374 ppm (nominal) Exposure duration: 6h/d, 5d/wk for 18 months, interim sacrifice at 12 months	**Positive: Malignant and benign liver tumours** **Carcinomas in male mice exposed by inhalation to 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 (1346 mg/m³)** 0, 15, 76, 374 ppm, equivalent to 54, 274, 1346 mg/m³, **males: liver carcinomas** 2/50, 2/50, 1/50, 10/50; statistically significant at 374 ppm (1346 mg/m³) 0, 15, 76, 374 ppm, equivalent to 54, 274, 1346 mg/m³, males: liver adenomas 4/50, 11/50, 10/50, 11/50, not statistically significant, within historical control range 0, 15, 76, 374 ppm, equivalent to 54, 274, 1346 mg/m³, females: liver adenomas 0/50, 0/50, 1/50, 3/50; not statistically significant **$LOAEC_{sys,\ m}$ = 15 ppm (54 mg/m³) for liver tumour development** **NOAEC not available** According to CLP butanone oxime fulfils the criteria for classification as **Carc. 1B, H350: May cause cancer**	**Key study** Test material: butanone oxime, purity: > 99.9 %	Newton et al. (2001); TL18 (1993), unpublished study report, confidential

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

No information is available.

4.10.1.2 Carcinogenicity: inhalation

There are combined chronic toxicity and carcinogenicity studies (similar to OECD TG 453/ EU B.33) in rats and mice available. In the carcinogenicity part of the studies F344 rats (50/sex/group) and CD-1 mice (50/sex/group) were exposed 6h/day, 5 days/week for 26 months (rats) or 18 months (mice) via whole-body inhalation exposures to target butanone oxime vapour concentrations of 0, 15, 75, and 374 ppm (corresponding to 0, 54, 270, and 1346 mg/m³). Satellite groups of rats and mice (10/sex/group/interval) were exposed for 3, 12, or 18 months (rats) or 12 months (mice) to evaluate chronic toxicity (Newton et al. 2001; TL18, 1993, 1994, unpublished study report, confidential). The age of the test animals was not reported but due to the long study duration and because the study was conducted similar to OECD TG 453, it is assumed that at exposure start mice and rats were – as preferred in such studies – around the age of weaning (4 – 10 weeks) (OECD TG 543, 2009). In both

studies butanone oxime was used as test material. The results of the combined chronic toxicity and carcinogenicity studies in rats and mice are summarised in Table 24 (see there).

Results regarding clinical laboratory studies (haematology, clinical biochemistry, urinalysis) and non-neoplastic microscopic findings observed in rats and mice are presented in 'Chapter 4.7 Repeated dose toxicity' (for more details see there).

Results from chamber monitoring showed that particle size distributions and mass concentrations of background particulate were similar among all the chambers including the air control, indicating that there was no measurable butanone oxime present as an aerosol. The chamber levels of methyl ethyl ketone, a possible hydrolysis product of butanone oxime, were less than 1 %.

The analyses of the survival rate in the rat and mice studies with butanone oxime found no statistically significant difference in survival among the exposure groups when compared to control. At termination of the rat study after treatment over a period of 26 months, in the control group the survival rate was 34 % in the males and 60 % in the females. At termination of the study in mice after 18 months, survival over all groups averaged 50 % in the male mice and 60 % in the females (see Table 25).

Table 25: Percent survival after exposure for 18 months in mice and 26 months in rats

Butanone oxime	**Mice (%)**		**Rats (%)**	
Exposure (ppm)	**Males**	**Females**	**Males**	**Females**
0	43	61	34	60
15	57	51	37	58
75	52	62	27	60
374	48	65	43	76

Results from the study in rats:

At termination of the rat study, mean body weights and body weight gains from study initiation were significantly elevated by exposure to butanone oxime in both the males and females. After 13 weeks of exposure, the 374 ppm males were 13 % heavier than the control males and the females were 4 % heavier.

Butanone oxime-related increases in absolute and relative organ weights were seen in the liver, spleen and testes. After treatment for 3 months in the 374 ppm group, the absolute liver weights in the males and females were elevated relative to the control group weights by about 23 and 13 %, respectively. The weight difference was still statistically significant in the males after 12 months. After 18 months no significant difference was noted. At study termination (26 months) the liver weights were again significantly elevated in males from the 374 ppm group (40 %) relative to control weights.

After treatment for 3 and 12 months, the absolute spleen weights in the 374 ppm group were elevated by about 33 % greater than the control group spleen weights in both males and females. By study termination (26 months) there was no significant difference in the spleen weights relative to control weights.

The absolute and relative testes weights were statistically significantly elevated in the 374 ppm group after 3, 18, and 26 months of treatment but not at 12 months. After treatment for 3 and 18 months the increase was small, but by study termination (26 months), the testes weights in the 374 ppm group were 82 % greater than that of the control males. There was no microscopic correlation associated with this increase in testes weights.

Treatment-related macroscopic findings were not observed after 3 or 12 months of treatment. At interim sacrifice after 18 months of treatment an increased incidence of red/tan discoloration of the liver was observed in 2/9 male and 2/10 female rats in the 75 ppm group and 6/9 male rats in the 374 ppm group. After 26 months of treatment tan to red discoloration of the liver occurred with greater incidence in males and females from the 374 ppm group. In the males at 374 ppm, an increased incidence of nodules/masses of the liver was observed.

Microscopic findings were noted in the spleen of rats after treatment with 374 ppm for 3 months. These were correlated to the increased organ weight an increased incidence of congestion. Pigment deposition in reticuloendothelial cells and extramedullary haematopoiesis also occurred with greater severity in the 374 ppm group males and females. After 12 months of treatment, there was an increase in the severity of congestion in the spleen of male and female rats of all test groups; this roughly correlated with increased spleen weights. In addition, there was an increase in the severity of pigment deposition in reticuloendothelial cells in males of the 75 and 374 ppm groups and an increase in the severity of extramedullary haematopoiesis in females in the 374 ppm group. After treatment of 18 months increased severity of congestion was observed in females at all tested concentrations, and greater severity of pigment deposition in reticuloendothelial cells and of extramedullary haematopoiesis in the 374 ppm exposure group. At terminal sacrifice (after 26 months) in females there was a statistically significant greater incidence of lymphoreticular mononuclear-cell leukaemia in the control group than in all test groups. In males this observation was limited to the 374 ppm test group. The previous findings in the spleen, e.g. congestion, extramedullary haematopoiesis, and pigment in reticuloendothelial cells could not be evaluated in those animals with leukaemia because the tumour cells effaced the architecture of the organ.

At 12 and 18 months sacrifice, the incidence and severity of basophilic foci and hepatocyte vacuoles in the liver were increased in males in the 374 ppm group. Also observed in the liver was a decrease in the incidence of hyperplasia/proliferation of the biliary duct in male and female rats in the 374 ppm group after 12 months of treatment and of males at the 18 months sacrifice, and further a decrease of peribiliary fibrosis in male rats in the 374 ppm group after 12 and 18 months of treatment. After 26 months of inhalation exposure to butanone oxime an increased number of animals with malignant and benign neoplasms in the liver were noted. In male rats statistically significant increased incidence of hepatocellular carcinoma occurred at 374 ppm and a concentration-related statistically significant increase in hepatocellular adenoma in male rats at 75 and 374 ppm compared to the concurrent controls. The comparison of the liver adenoma incidence of male F344 rats at 15 ppm (2/51, 4 %) with data from the historical control data base of the same strain and test laboratory has shown that the incidence at 15 ppm was within the historical control range of liver adenoma in F344 rats in this test laboratory (range of 3 - 4 %). However, specific details on historical control data of this species and laboratory, such as incidences of specific tumour types in control animals, were not available. Data of the NTP Historical Controls Report (2010) on F344 rats determined a mean percentage of occurring liver carcinomas of 1.01 % and 0.33 % for male and female control rats, respectively, after chronic inhalation exposure (clean air control).
In the study by Newton et al. (2001), an increase in hepatocellular adenoma (not statistically significant; in historical range lab controls) was also found in female rats. An overview of neoplastic changes in the liver of male and female test and control rats after 26 months of treatment, as well as historical control data reported in the NTP Historical Controls Report (2010) are given in Table 26. The overview compromises animals killed at terminal sacrifice and all unscheduled deaths.

Table 26: Neoplastic changes in the liver of rats (male/female), 26 months treatment, and historical control data (HCD) from the NTP Historical Controls Report (2010) on F 344 rats after chronic inhalation exposure to clean air

Concentration	HCD	0	15 ppm	75 ppm	374 ppm
Adenoma, male	1/299 (0.33 %)	0/50 (0 %)	2/51 (3.9 %)	**5*/51 (9.8 %)**	**18**/51 (35.3 %)**
Adenoma, female	1/300 (0.33 %)	0/50 (0 %)	0/50 (0 %)	2/50 (4.0 %)	4/50 (7.8 %)
Carcinoma, male	3/299 (1.0 %)	0/50 (0 %)	0/51 (0 %)	1/51 (2.0 %)	**12**/51 (23.5 %)**
Carcinoma, female	1/300 (0.33 %)	0/50 (0 %)	0/50 (0 %)	0/50 (0 %)	0/50 (0 %)
Combined adenoma and carcinoma (m/f)	0.33/0.17 (normalised to 50 animals)	0/0	2/0	**6**/2**	**27**/4**

*Mean value significantly different from concurrent control at $p \leq 0.05$; ** Mean value significantly different from control at $p \leq 0.01$

Further findings in the liver were an increase in the incidence of basophilic foci in males and females at 374 ppm (with increased severity in males at 15, 75 and 374 ppm; and in females at 374 ppm), a slight increased incidence of spongiosis hepatis in males at ≥ 15 ppm (with an increased severity at 75 and 374 ppm), and slight increases in the incidence of intracytoplasmic vacuoles in males at 75 and 374 ppm and in females at 374 ppm. There was a decrease in the incidence of two findings in the liver of males and females at 374 ppm, these were peribiliary fibrosis and hyperplasia/proliferation of the biliary duct.

At the terminal sacrifice (26 months exposure by inhalation) an increased incidence of mammary gland fibroadenomas was noted in male and female rats compared to the concurrent controls. Tests of statistically significance for mammary gland fibroadenomas showed a statistically significant dose response ($p < 0.05$) and differences between control and high dose groups in male rats at 374 ppm. An increase of mammary gland fibroadenomas was also observed in females, but achieved no statistically significance. Historical control data was not available for this tumour type, species and laboratory, but values obtained from the NTP Historical Controls Report (2010) for this tumour type and species are reported in Table 27, together with the incidences of mammary gland fibroadenomas of male and female test and control rats determined in the current study.

Table 27: Number of mammary gland fibroadenomas in rats, 26 months treatment, and historical control data (HCD) of the NTP Historical Controls Report (2010) on F 344 rats after chronic inhalation exposure to clean air (normalised to 50 test animals)

Concentration	HCD	0	15 ppm	75 ppm	374 ppm
Fibroadenoma, male	1.17/50	2/50	2/51	4/51	9/51*
Fibroadenoma, female	23/50	10/50	7/50	9/50	17/50

*Mean value significantly different from the concurrent control at $p \leq 0.05$

It is concluded that long-term exposure by inhalation to butanone oxime caused tumour development in the liver of male and female rats. There was a significantly increased incidence of liver carcinomas and adenomas in male rats at 75 and 374 ppm compared to the concurrent controls and historical control data (NTP Historical Controls Report, 2010). In female rats an increase in hepatocellular adenoma was also noted, but the difference to controls did not achieve statistical significance. The incidence of fibroadenomas in the mammary gland was significantly increased in male rats at 374

ppm (actual concentration of 1346 mg/m^3) compared to the concurrent controls and historical control data reported in the NTP Historical Controls Report (2010). Therefore it was concluded that under the exposure conditions of this study, butanone oxime is a carcinogen in F344 rats.

Results from the study in CD-1 mice:

Mean body weights and body weight gains were not significantly affected by exposure to butanone oxime.

At the 12 months interim sacrifice, significant effects in mouse organ weights were noted in the liver. In the females there was a dose-related increase with statistical significance in the 374 ppm group with a 17 % increase in the liver/brain weight ratios. In the males, there was a 12 % increase in the liver/brain weight ratio that was not statistically significantly different. At termination of the study after exposure duration of 18 months, there was no butanone oxime-related effect on absolute or relative organ weights.

There were no butanone oxime-related macroscopic findings at the 12 month interim sacrifice. Microscopically, changes were seen in the liver indicating hepatotoxicity, and occurred with greater incidence primarily in animals exposed to 75 and 374 ppm. These changes consisted of centrilobular hepatocellular hypertrophy and necrosis. No butanone oxime-related tumour development was noted after one year exposure. Although occurring in all groups including controls, liver changes appeared with greater incidence in the animals from the 374 ppm group after 18 months of treatment. These changes consisted of centrilobular hepatocellular hypertrophy, pigment deposition in the reticuloendothelial cells, necrosis, and granulomatous inflammation. An increased incidence of liver adenomas and carcinomas primarily in male mice was seen in the 374 ppm group compared to the concurrent controls and to data of a study on spontaneous tumour formation in the same species (Maita et al., 1988). An overview of neoplastic changes in the liver of male and female mice exposed to butanone oxime and of concurrent control mice is given in Table 28.

Table 28: Neoplastic changes in the liver of mice, 18 months exposure, and results of a study by Maita et al. (1988), examining the spontaneous tumour formation (STF) in CD-1 mice (normalised to 50 test animals)

Butanone oxime concentration	**STF**	**0**	**15 ppm**	**75 ppm**	**374 ppm**
Number examined (male/female)	50/50	50/50	50/50	50/50	50/50
Adenoma (m/f)	13.2/2.6	4/0	11/0	10/1	11/3
Carcinoma (m/f)	4.5/0.5	2/0	2/0	1/0	**10*/0**
Combined adenoma and carcinoma (m/f)	8.9/0.03	6/0	13/0	11/1	**18**/3**

*Mean value significantly different from control at $p \leq 0.05$; ** Mean value significantly different from control at $p \leq 0.01$

In male mice, there was an increase in tumour induction in the liver and a decrease in latency for liver carcinomas in the 374 ppm group, relative to the control, 15 and 75 ppm groups. There was a slight increase in the incidence of liver adenoma in females from the 374 ppm group. However, the increase was not statistically significant. Historical control data for this species and laboratory was not available, but results of a study (Maita et al. 1988) examining the spontaneous tumour formation (STF) in CD-1 mice also support these findings (Table 28).

It was concluded that under the exposure conditions of this study, butanone oxime is a carcinogen in CD-1 mice.

Overall in combined chronic toxicity and carcinogenicity studies in rats and mice exposed by inhalation to vapours of butanone oxime sufficient evidence of animal carcinogenicity was demonstrated. Two animal experiments using two species (rat and mouse) resulted in clear evidence for carcinogenicity. A causal relationship has been established between butanone oxime and a statistically significant increased incidence of a combination of benign and malignant tumours in well-conducted studies. Being similar to OECD TG 453/EU B.33 both studies are well conducted and do not cast doubts about the relevance of the results. Tumour development was noted by inhalation of relative low concentrations in rats and mice. Butanone oxime caused an increased incidence of liver tumours in both species. There was an increased incidence of malignant and benign liver tumours in both sexes of rats and mice at all tested exposure concentrations. In both species, the liver tumours appeared relatively late in the life of the animals, with no significant increase in tumours after 12 months of exposure in mice and after 18 months of exposure in rats. Lifespan shortening was not observed. The incidence of liver carcinomas was significantly increased in male rats and mice. The female rats and mice showed no increased incidence of liver carcinomas. A LOAEC of 15 ppm (54 mg/m^3) for carcinogenicity (liver tumour development) was derived for rats and mice. A statistically significantly increased incidence of mammary gland fibroadenomas was also observed in male rats at the high concentration of 374 ppm. A NOAEC for carcinogenicity was not derived for the rat and also not for the mouse.

4.10.1.3 Carcinogenicity: dermal

No information is available.

4.10.2 Human information

No information is available.

4.10.3 Other relevant information

No information is available.

4.10.4 Summary and discussion of carcinogenicity

Data on carcinogenicity of butanone oxime was obtained from animal testing conforming to internationally agreed test guidelines. Carcinogenicity studies on butanone oxime have been conducted in rats and mice using the inhalation route of exposure. There are no epidemiological studies available which demonstrate that butanone oxime induced cancer in humans.

Evidence from animal experiments

The carcinogenic potential of butanone oxime has been studied in two combined chronic toxicity and carcinogenicity studies and in two species. Butanone oxime was administered by whole-body inhalation as a vapour for 6h/day, 5 days/week for 26 months to F344 rats and 18 months to CD-1 mice, and both sexes each. Satellite groups of rats and mice (10/sex/group/interval) were exposed for 12 months (mice) and 3, 12, or 18 months (rats) to evaluate chronic toxicity. Studies using the oral or the dermal route of exposure are not available.

There is sufficient evidence of carcinogenicity in experimental animals. Carcinogenic potential of butanone oxime was demonstrated for the inhalation route of exposure. The evidence of

carcinogenicity is based on well documented animal experiments in rats and mice. The combined chronic toxicity and carcinogenicity studies in rats and mice (similar to OECD TG 453/EU B.33) have demonstrated that butanone oxime causes liver tumours (adenomas and carcinomas) in both species at all tested exposure concentrations. However, statistically significant increases in incidence were observed at 75 ppm (270 mg/m^3) and 374 ppm (1346 mg/m^3) for liver adenomas in male rats and at 374 ppm (1346 mg/m^3) for liver carcinomas in male rats and mice. An increased incidence of liver adenomas occurred also in female rats and mice at 270 and 1346 mg/m^3, but was not statistically significant. A dose-response relationship for tumour induction in the liver of rats and mice was observed in both sexes. The incidence of fibroadenomas in the mammary gland was also significantly increased in male rats at 1346 mg/m^3.

These available data from long-term inhalation studies with butanone oxime in rats and mice have provided clear evidence that butanone oxime induced cancer in both species. Butanone oxime induced malignant and benign tumours in the liver of rats and mice in well performed experimental studies.

Since 2000, butanone oxime is classified as carcinogen Category 2 and is listed in Annex VI of CLP as Carc. 2; H351. It is assumed that the reported study results for carcinogenicity do not comply with the legal classification of butanone oxime as carcinogen Category 2. Based on the available data on carcinogenicity butanone oxime fulfils the criteria for classification and labelling as Category 1B carcinogen, H350 according to CLP.

Human data

There are no human data on butanone oxime-induced carcinogenicity available.

Germ cell mutagenicity data

Butanone oxime has been tested regarding genotoxicity in a variety of systems including rat hepatocytes and an in vivo peripheral blood micronucleus test with an administration period of 90 days. The results from mutagenicity or genotoxicity tests in vitro and in vivo were mostly negative, including bacterial mutagenicity, unscheduled DNA-synthesis in primary rat hepatocytes, micronucleus tests in rats and mice, and an in vivo study that utilized inhalation exposure and was found to be negative for DNA adducts in rat liver cells.

Mode of action (MoA)

The modes of action for butanone oxime induced **liver tumours in rats and mice** following long-term exposure by inhalation have not yet been identified.

For butanone oxime the results from mutagenicity or genotoxicity tests in vitro and in vivo were mostly negative (see above ‘Germ cell mutagenicity data’).

Findings obtained from related compounds for carcinogenicity may give indications of a likely mechanism of the observed liver carcinogenicity of butanone oxime. As a possible mechanism for the butanone oxime-induced hepatocarcinogenicity in rats and mice the noted bioactivation of ketoximes is discussed. Ketoximes are known to undergo NADPH-dependent liver microsomal metabolism to nitric oxide/nitrogen monoxide (· NO) and ketones. In addition, nitric oxide synthase can catalyse the oxidative denitration of the >C=N-OH group of amidoximes. Mechanistically, the reaction is believed to proceed via a transient, cytochrome P450-catalyzed conversion of their >C=N-

function to a peroxide (Caro et al. 2001). For the related compound acetoxime (acetone oxime; $(CH_3)_2C=N-OH$; CAS 127-06-0) as a possible mechanism for activation the oxidation to the hepatocarcinogen 2-nitropropane (CAS 79-46-9; classified as Carc. 1B, H350; Index No 609-002-00-1) is postulated: $(CH_3)_2C=NOH + O \rightarrow (CH_3)_2CHNO_2$ (Mirvish et al. 1982). Correspondingly, it is assumed that butanone oxime will already be metabolically activated to reactive intermediates in the carcinogenesis. Toxicokinetic studies have shown that butanone oxime is extensively metabolized, yielding CO_2, methyl ethyl ketone, glucuronides, and other polar metabolites (Burka et al. 1998). Three metabolic pathways for butanone oxime could be distinguished: Hydrolysis to 2-butanone, oxidation to butane 2-nitronate, and a possibly third reductive pathway (Janku et al. 2000). Oxidation of butanone oxime to a carcinogenic agent (e.g., nitronates of secondary-nitroalkanes which are mutagenic and tumourigenic in rodents) mediated by sulfotransferase (cytosolic enzyme) has been also postulated to play a role in the MoA for the liver tumourigenicity of butanone oxime. A similar MoA has been assumed which is mechanistically described for another secondary nitroalkane 2-nitropropane. For the genotoxic hepatocarcinogen 2-nitropropane, an activation pathway involving sulfontransferases to give acetoxime *O*-sulfonate is assumed. The genotoxicity of 2-nitropropane in rats has been attributed to aryl sulfontransferase-mediated formation of DNA-reactive nitrenium ions from the anionic form of 2-nitropropane, propane 2-nitronate (Sodum et al. 1993; Sodum et al. 1994; Sodum and Fiala 1997, 1998; Kreis et al. 2000). 2-Nitropropane produces characteristic base modifications in rat liver RNA and DNA, including the amination and the oxidation of C8 of guanine (Fiala et al. 1995). The ultimate reactive metabolite postulated in the pathway is hydroxylamine *O*-sulfonate which aminates/oxidizes nucleosides through a nitrenium ion intermediate. Hydroxylamine *O*-sulfonate is a presumed product of the hydrolysis of acetoxime *O*-sulfonate formed postulated to be formed from 2-nitropropane/propane 2-nitronate by sulfate conjugation.

In experiments comparing the oxidation of butanone oxime and acetoxime it was shown that the incubation of liver microsomes from mice, rats and several human liver samples with butanone oxime resulted in the formation of nitronates, but at very low rates. No sex and species differences in the rates of microsomal oxidation of butanone oxime to butane 2- nitronate were observed (Völkel et al. 1999).

From the available data, it appears that the biotransformation of butanone oxime in the tumourigenicity is complex with many interacting steps of several enzymes potentially involved. Further it is considered that one of several possible mechanisms for the increased incidences of liver tumours in rats and mice may be the metabolism of butanone oxime to reactive intermediates (e.g., nitronates), mediated by sulfontransferase. A number of other factors and mechanisms for the tumour response of butanone oxime may be also involved.

No effects of butanone oxime on hepatic peroxisome proliferation and on serum testosterone levels were observed in male F344 rats after oral treatment for 4 weeks (TL14, 1995a, unpublished study report, confidential).

No species-specific mode of action for butanone oxime carcinogenesis was identified; as a default the tumour responses in rats and mice are considered as relevant for the man.

In summary, the negative genotoxicity studies with butanone oxime support the adoption that the hepatocarcinogenicity of butanone oxime in rats and mice is, inter alia, is attributed to other/further mechanisms. At present it is expected that tumour initiation in the liver of rats and mice is induced by an interaction of activated butanone oxime-metabolite(s) with nucleic acids, and enhanced tumour development following cytotoxic effects of butanone oxime in the liver. Due to uncertainties in the available information it is not possible to reach a final conclusion regarding likely modes of action of hepatocarcinogenicity in experimental animals. Accordingly, it is assumed that the potential

mechanisms behind butanone oxime carcinogenesis in the liver of rats and mice after long-term exposure by inhalation are highly complex involving a number of events and factors which are still unknown.

The modes of action for butanone oxime induced **mammary gland tumours in male and female rats** following long-term exposure by inhalation have not been identified until now.

Significantly increased incidences of fibroadenomas in the mammary gland were only seen in male rats exposed to 374 ppm butanone oxime. At termination of the long-term study an increase of mammary gland fibroadenomas was also observed in female rats, but achieved no statistically significance. From the scientific literature it is known that several factors greatly influence the susceptibility, magnitude and type of neoplastic response, and growth rate of mammary neoplasms in the rat. These include genetic factors, degree of differentiation of mammary gland at time of chemical exposure, physiological and hormonal status, and diet.

There is no indication in the available investigations that the determined carcinogenicity in rats and mice has no relevance to humans.

Conclusion in respect to carcinogenicity

There is clear evidence that butanone oxime induced carcinogenicity in rats and mice after long-term exposure by inhalation. Carcinogenicity in animals is derived from two independent studies, carried out at different times. In two well performed experiments it was shown that butanone oxime induced malignant and benign tumours in the liver of the two species: rats and mice.

Butanone oxime-induced tumour development in the liver is observed in rats and mice at all tested exposure concentrations (≥ 15 ppm; actual concentration of 54 mg/m^3). However, statistically significant increases in incidence were observed only at the mid and high concentration of 270 and 1346 mg/m^3 for liver adenomas in male rats and at 1346 mg/m^3 for liver carcinomas in male rats and mice. An increased incidence of liver adenomas compared to the concurrent controls occurred also in female rats and mice at 270 and 1346 mg/m^3, but was not statistically significant. A dose-response relationship for tumour induction in the liver of rats and mice was observed in both sexes. A LOAEC of 15 ppm (54 mg/m^3, lowest concentration tested in the study) for carcinogenicity (liver tumour development) was derived for rats and mice.

A statistically significantly increased incidence of mammary gland fibroadenomas was also observed in male rats at the high concentration of 1346 mg/m^3.

Under the conditions described in the combined chronic toxicity and cancerogenicity studies a NOAEC for carcinogenicity was not derived for the rat and also not for the mouse.

4.10.5 Comparison with criteria

Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies in animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

For the purpose of classification for carcinogenicity, substances are allocated to one of two categories. Classification of a substance as a carcinogen in Category 1A and 1B is based on consideration of the strength of the evidence of available data for classification with considerations of all other relevant information (weight of evidence) being taken into account as appropriate. In certain instances, route-

specific classification may be warranted, if it can be conclusively proven that no other route of exposure exhibits a hazard.

Hazard categories for carcinogens:

'Category 1: Known or presumed human carcinogens

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A or 1B.'

'Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

'Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in Category 1A or 1B is based on strength of evidence together with additional considerations (s. section 3.6.2.2 of CLP). Such evidence may be derived from:

- Human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
- Animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen).'

Butanone oxime is possibly carcinogenic to humans. But there are no data available of cancer in humans related to butanone oxime exposition. Therefore classification as Category 1A carcinogen is not appropriate.

The decision of classification of a substance in Category 1B based on animal experiments means a causal relationship has been established between the agent and an increased incidence of malignant neoplasm's or of an appropriate combination of benign and malignant neoplasms in

a) two or more species of animals or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols;

b) in both sexes of a single species;

c) occurrence of malignant neoplasm to an unusual degree with regard to the incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

In comparison to the given criteria for CLP butanone oxime fulfils the criteria for Category 1B carcinogen.

There is sufficient evidence of carcinogenicity from well performed experimental studies on animals. Butanone oxime caused tumours in two rodent species carried out independently.

Butanone oxime induced cancer in both sexes when administered by whole-body inhalation as a vapour 6h/day, 5 days/week for a period of 26 months to F344 rats and 18 months to CD-1 mice.

Tumours induced by butanone oxime were found in the liver (malignant and benign) in rats and mice and in the mammary gland (benign) in rats.

Tumours in the liver (adenomas and carcinomas) occurred in both species at all tested exposure concentrations (15, 75, 374 ppm, equivalent to 54, 270 and 1346 mg/m^3). Statistically significant increases in incidence were observed at the mid and high concentration for liver adenomas in male rats and at the high concentration for liver carcinomas in male rats and mice compared to the respective control groups. An increased incidence of liver adenomas compared to the concurrent controls occurred also in female rats and mice at the mid and high concentration, but was not statistically significant. A statistically significantly increased incidence of mammary gland fibroadenomas was also observed in male rats at the high concentration (374 ppm, equivalent to 1346 mg/m^3).

A dose-response relationship for tumour induction in the liver was observed in both species, and in the mammary gland in rats only.

'Category 2: Suspected human carcinogens

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on the strength of evidence together with additional considerations (s. section 3.6.2.2 of CLP). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.'

Following consideration would lead to classification as Category 2:

a) the evidence is limited to a single experiment;

b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies;

c) the agent increases the incidence only of benign neoplasm or lesions of uncertain neoplastic potential; or

d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

Category 2 is not appropriate because the results from the available data do not match the criteria for Category 2 classification. The evidence is neither limited to a single experiment, nor limited with regard to benign neoplasm, or promoting activity.

In conclusion, the available data for carcinogenicity of butanone oxime does not comply with the legal classification of butanone oxime as carcinogen Category 2. Butanone oxime rather fulfils the criteria for classification and labelling as Category 1B carcinogen, H350 according to CLP.

4.10.6 Conclusions on classification and labelling

According to CLP butanone oxime has to be classified as:

Carc. 1B and labelled with hazard statement H350: May cause cancer; with the pictogram "GHS08: Health hazard", and with the signal word "Danger".

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Combined chronic toxicity and carcinogenicity studies are available in rats and mice. F344 rats and CD-1 mice were exposed to butanone oxime for 6 h/day, 5 days/week for 26 months (rats) or 18 months (mice) via whole-body inhalation at vapour concentrations of 0, 15, 75 or 374 ppm (corresponding to 0, 54, 270 and 1 346 mg/m^3).

At study termination, tumour development was observed in the livers of rats and mice at all tested concentrations, which followed a dose-response relationship. However, statistically significant increases in incidence were observed only at the mid and high concentrations of 270 and 1 346 mg/m^3 for liver adenoma in male rats and at 1 346 mg/m^3 for liver carcinoma in male rats and mice. An increase in liver adenomas compared to the concurrent controls occurred in female rats and mice at 270 and 1 346 mg/m^3, but this was not statistically significant.

Also observed was a statistically significantly increased incidence of fibroadenoma in the mammary gland of male rats dosed with 1 346 mg/m^3.

In conclusion, there is sufficient evidence of carcinogenicity from two well-performed experimental studies in two species. Butanone oxime induced tumours in the liver (benign and malignant) in rats and mice and in the mammary gland (benign) of rats. These tumours occurred in the presence of a dose-response relationship. Therefore, as there were treatment-related tumours found in two species, in two separate experiments, the DS proposed that butanone oxime fulfils the criteria for classification and labelling as a category 1B carcinogen; H350.

Following a comment made during the public consultation, the Dossier Submitter further provided an assessment of the carcinogenic potency of butanone oxime and considered whether there was any scope for setting a specific concentration limit (SCL). Based on the lowest exposure concentration at which there was a significant increase in tumour development in rats, a T25 value of 492 mg/m^3 (6h/day) was derived. This equated to an oral dose of approx. 108.3 mg/kg bw/day butanone oxime. According to CLP guidance, the Dossier Submitter observed that a T25 value of > 100 mg/kg bw/day may indicate a carcinogen of low potency and support the setting of a specific concentration limit of 1.0%.

Comments received during public consultation

This aspect of the CLH dossier attracted the most comments. Seven comments were made by or on behalf of industry and three were made by Member States.

The comments from industry were all in opposition of the proposed classification as Carc. 1B. A recurring theme was that there had been no evidence of butanone oxime carcinogenicity in humans in spite of extensive and widespread use of this substance. There were no grounds to justify a further evaluation of this endpoint in the absence of any new studies or other evidence; the classification should remain as Carc. 2. The absence of a

plausible mode of action weakened the case for a Carc. 1B classification for some commenters. Detailed comments were provided to support the view that butanone oxime was not a genotoxic carcinogen.

The three MS that commented all agreed with the proposed classification. However, one questioned the sex-specificity of the findings in rats and mice and requested more information on this. Another MS noted the relatively low exposure levels that had led to carcinogenic responses in rats and requested an assessment of carcinogenic potency and the possibility of setting a SCL for this endpoint.

Assessment and comparison with the classification criteria

The classification proposal is based on the findings in two combined chronic toxicity and carcinogenicity studies: one in rats (first reported in 1994) and one in mice (1993). Both studies were carried out similarly to OECD test guideline 453 and to GLP.

Rats

F344 rats (80/sex/dose in the main carcinogenicity study and 10/sex/dose sacrificed at intervals of 3, 12 and 18 months) were exposed to butanone oxime (> 99.9 % purity) by whole-body vapour exposure at doses of 0, 15, 75 or 374 ppm (equivalent to 0, 54, 270 or 1 346 mg/m^3 in both males and females) for 6 h/day, 5 days/week for an extended period of 26 months.

Mortality rates at the end of this study are shown below. It's possible that the extended period of the study may have contributed to these death rates. However, no information is available on the times that deaths occurred.

	% of rats surviving to 26 months (end of study)	
Concentration in mg/mg^3	**Males**	**Females**
0	34	60
54	37	58
270	27	60
1 346	43	76

The relatively high mortality seen in males was not treatment-related and was not considered to diminish the relevance of the carcinogenicity findings.

At the end of the study, there was an increased incidence of benign and malignant liver tumours in males and an increase of benign liver tumours only in females. In males, the increased incidence of adenoma showed a dose-response relationship, whereas an increase in carcinoma was seen only at the top dose. Only in males did these increases in tumours reach statistical significance.

	Liver tumour incidence after 26 months **Butanone oxime concentration (mg/m^3)**			
	0	54	270	1 346
Males, adenoma	0/50 (0 %)	2/51 (3.9 %)	**5/51* (9.8 %)**	**18/51** (35.3 %)**
Males, carcinoma	0/50 (0 %)	0/51 (0 %)	1/51 (2.0 %)	**12/51** (23.5 %)**

Females, adenoma	0/50 (0 %)	0/50 (0 %)	2/50 (4.0 %)	4/50 (7.8 %)
Females, carcinoma	0/50 (0 %)	0/50 (0 %)	0/50 (0 %)	0/50 (0 %)

No laboratory control data available
* $p \leq 0.05$ ** $p \leq 0.01$

The CLH report included the test laboratory historical control data only for liver adenoma in male F344 rats (3-4 %). It's not clear how many studies this data was derived from, when they were conducted, or whether they were all of 26-months duration, so this rate for liver adenoma is not very informative. There are also control data for this rat strain available from the US NTP Historical Controls Report (2010). Although again this may not relate to a study duration of 26 months, the implication is that liver tumours generally are not common in rats following chronic inhalation exposure of clean air.

Liver tumours	Laboratory HCD	NTP Historical Controls Report 2010 HCD
Males, adenoma	3 – 4 %	1/299 (0.33 %)
Males, carcinoma	ND	3/299 (1.0 %)
Females, adenoma	ND	1/300 (0.33 %)
Females, carcinoma	ND	1/300 (0.33 %)

ND = No data

There were no specific non-neoplastic findings in the livers of the rats that could account for the tumours seen. In the highest exposure group males only, there was a 40 % increase in mean liver weight (relative to the control group), and in males and females, there were greater incidences of tan/red discolouration of the liver. Slight increases in the incidence of intracytoplasmic vacuoles were noted in males in the mid and highest exposure groups and in females of the top dose group. There was also an increase in the incidence of basophilic foci in hepatocytes in males and females compared to controls, with an increase in severity in males in all treatment groups and in females, at the top dose only.

There was a slight increase in incidence of spongiosis hepatis in all treatment groups of malesat the end of the study, with increased in severity at 270 and 1 346 mg/m^3. Spongiosis hepatis represents a degenerative change and may be seen in normal hepatic parenchyma as well as in proliferative hepatocellular lesions such as foci and neoplasms.

In addition to the liver tumour findings, there was also an exposure-related increase in the incidence of benign fibroadenoma in the mammary glands of male rats at the highest two exposure levels. This reached statistical significance in the highest exposure group. A small increased incidence of the same tumour type was seen in females at the highest exposure level only, but this was not statistically significant.

	Tumour incidence after 26 months **Butanone oxime concentration (mg/m^3)**			
	0	54	270	1 346
Fibroadenoma in males	2/50 (4 %)	2/51 (3.9 %)	4/51 (7.8 %)	**9/51* (17.6 %)**
Fibroadenoma in females	10/50 (20 %)	7/50 (14 %)	9/50 (18 %)	17/50 (34 %)

No laboratory control data available
* $p \leq 0.05$

No laboratory historical control data were provided for benign mammary gland tumours in rats. The US NTP Historical Controls Report (2010) shows that fibroadenoma in females, but not in males, appear to occur spontaneously at a relatively high rate following chronic

inhalation exposure of clean air. In males, the NTP report provides a value of 2.3 % for mammary gland fibroadenoma. This is a considerably lower rate than that seen in the two highest butanone oxime exposure groups which, in spite of the extended duration of this study, would appear to suggest that the findings in males were treatment-related.

Finding	NTP Historical Controls Report 2010 HCD
Fibroadenoma in males	1.17/50 (2.3 %)
Fibroadenoma in females	23/50 (46 %)

However, there were no non-neoplastic findings in this study in the mammary glands of males or females. Further, there were no indications of any treatment-related effects to the reproductive system in the reproductive and developmental toxicity studies provided. As such, the possibility that the observed tumours had occurred by chance, in the later stages of the extended lives of these animals, also cannot be ruled out. Specific information about the time of tumour development is not available, therefore this remains unclear.

There were no other tumour findings in rats. At the end of the study, in addition to the liver, non-neoplastic effects were seen in the olfactory epithelium of both males and females and testes of males. Throughout the study, effects on the blood indicative of anaemia were also observed. These other findings are described in detail under Specific Target Organ Toxicity: Repeated Exposure. They are not considered to have influenced the tumourigenic potential of butanone oxime in this study. The findings were not of such severity to suggest that the MTD had been exceeded in this study.

Mice

CD-1 mice (60/sex/dose in the main carcinogenicity study and 10/sex/dose sacrificed at an interval of 12 months) were exposed whole body to butanone oxime (> 99.9 % purity) vapour at concentrations of 0, 15, 75 or 374 ppm (equivalent to 0, 54, 274 or 1 346 mg/m^3 in both males and females) for 6h/day, 5 days/week for 18 months.

There was no effect on mortality in this study, survival rates in all groups averaged 50 % in males and 60 % in females. Mean body weights and body weight gains were not affected by treatment with butanone oxime. At study termination, there were no effects on absolute or relative organ weights.

At the end of the study, there was a statistically significant increase in incidence of liver cell carcinoma in male mice at the top dose only (20 % versus 4 % in controls). There was no evidence of liver carcinoma in females, however a slight increase in liver adenoma was noted in the top two dose groups (2 % and 6 % at 274 and 1 346 mg/m^3 respectively, versus 0 % in controls). These increases in benign tumours were not statistically significant.

	Liver tumour incidence after 18 months Butanone oxime concentration in mg/m^3			
Finding in CD-1 mice	0	54	274	1 346
Males, adenoma	4/50 (8 %)	11/50 (22 %)	10/50 (20 %)	11/50 (22 %)
Males, carcinoma	2/50 (4 %)	2/50 (4 %)	1/50 (2.0 %)	**10/50* (20 %)**
Females, adenoma	0/50 (0 %)	0/50 (0 %)	1/50 (2 %)	3/50 (6 %)
Females, carcinoma	0/50 (0 %)	0/50 (0 %)	0/50 (0 %)	0/50 (0 %)

No laboratory control data available
* $p \leq 0.05$

Historical control data for this species from the same laboratory were not available. The Dossier Submitter provided details of a publicly available study (1988) which examined spontaneous tumour formation in CD-1 mice following clean air exposure in lifetime studies. The findings from this study indicate that the increase of carcinoma in males of 16 % may exceed the natural formation of this type of tumour, following exposure to clean air inhalation. The finding of adenoma in females at the top dose (6 % versus 0 % in controls) was only marginally above the spontaneous adenoma formation seen in the literature (5.2 %).

Finding in CD-1 mice	Publically available historical control data for liver tumours
Males, adenoma	13.2/50 (26 %)
Males, carcinoma	4.5/50 (9 %)
Females, adenoma	2.6/50 (5.2 %)
Females, carcinoma	0.5/50 (1 %)

Findings indicative of liver toxicity occurred in both males and females of the high exposure group. There was an increased incidence of granulomatous inflammation (males: 43 % affected, versus 24 % in controls, and females: 43 % affected, versus 32 % in controls) and centrilobular hypertrophy (percentages and severity not given in the CLH dossier or the REACH Registration dossier). There was a slight increase in necrosis which occurred in females only of the top dose group.

There were no other tumour findings in mice. At the end of the study, in addition to the liver, non-neoplastic effects were seen in the olfactory epithelium of both males and females. Similar to rats, effects on the blood indicative of anaemia were observed throughout the study. Details are provided in the section Specific Target Organ Toxicity – Repeated Exposure. Overall, it is considered that the MTD was not exceeded in this study.

Conclusions regarding carcinogenic hazard

Overall, 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.

Comparison with criteria

As there are no epidemiological studies to inform on butanone oxime carcinogenicity to humans, classification in category 1A is not appropriate. However, the absence of reports of increased cancer rates amongst workers who exposed to butanone oxime is not a sufficient basis to conclude that this substance is non-carcinogenic to humans. In order to decide on the most appropriate classification, both the strength and the weight of the available evidence from the studies in rats and mice should be addressed.

The strongest evidence of a carcinogenic effect following exposure to butanone oxime is provided by the inhalation studies showing increased liver tumours in exposed male rats and male mice. No such tumours were seen in control animals. These observations, common to both species, provide relatively strong evidence of a causal relationship between butanone oxime exposure and a carcinogenic response. Whereas an increased tumour rate was only seen at the highest butanone oxime exposure level employed in the mouse study, it was seen in the mid and high exposure groups in rats. Female rats and female mice appeared less sensitive to this hepatocarcinogenic effect of butanone oxime. The evidence is considerably weaker in females, with only a small increase in (benign) adenoma at the top dose (in female mice and female rats), observed in the absence of statistical significance.

The increased incidence of fibroadenoma in the mammary gland of rats provides weak evidence of a carcinogenic effect. A clear increase in these benign tumours was observed in males of the highest exposure group. A slight increase was observed in females at this exposure level, however there was no dose response relationship and there was no statistical significance.

As the data on carcinogenicity were from two different studies, in two different species, and there are no unresolved questions regarding the adequacy of the design, conduct or interpretation of these studies, there is no reason to consider the strength of evidence to be limited. Generally, this is sufficient to justify classification of a substance in category 1B for carcinogenicity.

However the weight of the evidence should also be considered, especially with respect to the relevance of the findings to humans. Important factors to consider are as follows:

a. Tumour type and background incidence
By default, carcinogenic effects in experimental animals are considered relevant to humans. If the tumour observed can be judged to be of no relevance to humans this can exclude the finding from classification.

Clear increases above generally established background rates for the tested strains were observed for liver adenoma and carcinoma in male rats and liver carcinoma in mice. Tumours of this nature observed in animals are relevant for human hazard assessment.

b. Multi-site responses
Malignant liver tumours were observed in two species (rats and mice). There was some indication of a multi-site response due to the increased incidence of benign fibroadenoma observed in male rats. However, this was statistically significant only at the top exposure concentration and a comparable effect was not seen in female rats or in mice. Therefore, the evidence to support a multi-site response is not strong.

c. Progression of lesions to malignancy
As malignant findings were observed in mice and both benign and malignant findings were observed in rats, there was clear evidence of progression to malignancy of liver tumours.

d. Reduced tumour latency
The tumours observed in rats and mice did not occur early on in the studies. Butanone oxime did not cause reduced tumour latency. The latency of tumour development often reflects the potency of a carcinogen although carcinogens that cause reduced latency would not be placed in a more severe hazard category.

e*. Whether responses are in single or both sexes*
Tumours seen specifically in one sex only may arise for two broad reasons, either because of the gender-specificity of the target tissue or a gender-specific mechanism of carcinogenic action. In general, effects observed in one sex of laboratory animal only may be less convincing than effects seen in both sexes, unless there is a clear patho-physiological difference consistent with the mode of action to explain the single sex response. With butanone oxime, malignant tumours were seen in males only, but not in a gender-specific tissues. In females, there were slight increases in benign tumours, observed at the top dose in the absence of statistical significance. It is possible that females were merely less sensitive than males to the effects of butanone oxime. No species-specific mode of action has been identified and there were no clear differences between histopathology results in males and females. 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.

f. Whether responses are in single species or several species
Positive responses in several species add to the weight of evidence that a chemical is a carcinogen. Clear increases in liver carcinoma were seen in rats and mice. This provides strong evidence of a carcinogenic effect and increases the level of concern.

g. Structural similarity or not to a chemical(s) for which there is good evidence of carcinogenicity
The Dossier Submitter did not identify any structurally similar carcinogens as part of the case for classification. None were presented in the public consultation.

h. Routes of exposure
Exposure to butanone oxime was via whole body inhalation. According to the guidance, providing the substance is absorbed by the given route and the tumours observed were

not limited to local neoplasms at the site of administration, then this is an appropriate physiological route of exposure considered directly relevant to humans.

A question was raised during the public consultation as to whether the total exposure could be higher than indicated if animals were also ingesting the chemical following grooming. As the study is being used to identify a hazard and not risk, this is not a relevant factor affecting the assessment.

i. Comparison of toxicokinetic parameters between test animals and humans
There is no suggestion of a unique metabolic pathway in rodents. The available toxicokinetic evidence provided in animals provides no clear reason to suspect that a different mode of metabolism may occur in humans. Therefore, the tumours found in rats and mice remain relevant to humans.

j. The possibility of confounding effect of excessive toxicity at test doses
The highest dose in a carcinogenicity assay should ideally reflect the maximal tolerated dose (MTD). Excessive toxicity exceeding the MTD can affect the carcinogenic response. For example, toxicity such as necrosis with associated regenerative hyperplasia can lead to tumour development as a secondary consequence unrelated to the intrinsic carcinogenic potential of the substance itself. Tumours occurring only at excessive doses associated with severe toxicity generally have a more doubtful potential for carcinogenicity in humans.

In the studies carried out in mice and in rats there were no treatment-related increases in the number of deaths. Body weights remained comparable to the control (if not higher than controls) throughout the study. In terms of the whole animal, there were no signs of excessive toxicity.

k. Mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression
Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action; genotoxic or non-genotoxic. From the available mutagenicity studies, there is good evidence to suggest that butanone oxime is not genotoxic.

No specific studies were carried out to investigate anaemia as a potential mode of action. There is a possible argument that the changes in blood parameters, indicative of anaemia, could led to increased pressure to the spleen and liver, and that this could increase cancer rates in the liver. Small changes to blood parameters were observed in male and females rats of the top exposure group at 3 and 12 months intervals, but these signs were found to be reversible by 18 months in males and by 26 months in females. In mice, changes to blood parameters were observed at 12 months. They occurred from the mid dose group upwards and appeared to affect females to a greater extent than males.

These changes 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. Therefore, it seems unlikely that blood toxicity was a factor in the hepatocarcinogenicity of butanone oxime.

In rats and mice there were signs of liver toxicity and, to a greater or lesser extent, this may have influenced the carcinogenic response to butanone oxime. In rats, there was an

increase in liver weight, in top dose males only. Discolouration and *slight* increases in the incidence of intracytoplasmic vacuoles and an increase in the incidence of basophilic foci in hepatocytes were observed in both males and females. Despite liver findings in both males and females, clear increases in adenoma and carcinoma were only seen in males. There was no evidence of necrosis, regenerative hyperplasia or severe liver toxicity in male or female rats.

In mice, centrilobular hepatocellular hypertrophy and granulomatous inflammation were seen in both males and females of the highest exposure group. A slight increase in incidence of necrosis was observed in female mice of the top dose group only. Necrosis indicates serious liver toxicity, however, no malignant tumours were observed in females and the very small (statistically insignificant) increase in adenoma in the highest exposure group shows limited evidence for cancer. It therefore seems unlikely that in this study such liver toxicity was a key precursor of liver tumours.

Therefore, 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 has been identified and, therefore, by default, the tumours observed are relevant to humans.

For the mammary gland tumours in male rats only, there are no indications of any plausible mode of action for butanone oxime carcinogenicity. Given also the absence of an effect in female rats, or in male or female rats, these findings are of a low level of concern and don't add to the weight of evidence for classification provided by the liver tumour findings.

The possible impact of these factors on the overall level of concern about the carcinogenic hazard of butanone oxime is summarised in the following table.

	Factor to be taken into consideration	**Overall level of concern. Classification**
a	Tumour type and background incidence	Benign and malignant liver tumours of relevance to humans. High concern - Cat 1B
b	Multi-site responses	Clear evidence in liver, limited/insufficient evidence in mammary gland. Concern - at least Cat 2.
c	Progression of lesions to malignancy	Malignant liver tumours. High concern - Cat 1B.
d	Reduced tumour latency	No evidence of high potency. At least Cat 2.
e	Whether responses are in single or both sexes	Strong evidence in males, evidence for an effect in females less clear. Concern - at least Cat 2.
f	Whether responses are in single species or several species	Strong evidence in rats and mice. High concern - Cat 1B.
g	Structural similarity or not to a chemical(s) for which there is good evidence of carcinogenicity	No structurally similar carcinogens identified. At least Cat 2.
h	Routes of exposure	Inhalation exposure route relevant. At least Cat 2.

i	Comparison of ADME between test animals and humans	No indications of relevant species differences in toxicokinetics. At least Cat 2.
j	The possibility of confounding effect of excessive toxicity at test doses	No confounding by excessive toxicity. At least Cat 2.
k	Mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression	Although some limited evidence that liver (cyto)toxicity may have been a factor in the liver cancer seen in rats and mice, a mode of action has not been established for butanone oxime. No basis to discount relevance to humans Concern - at least Cat 2.

As shown, concern is raised by the clear laboratory evidence for the induction of tumours, the nature of the tumours, their relevance to humans, and the sensitivity of both species tested. All these factors provide a convincing profile for the carcinogenicity of butanone oxime and, in line with the CLP criteria, support a category 1B classification for butanone oxime. In the absence of any clear reason to lower the level of concern, it is not possible to justify a lower level of classification.

As one Member State commented during the public consultation, the available toxicokinetic information indicates that butanone oxime is readily absorbed following oral and dermal exposure. Therefore no route specific labelling should be introduced, as it cannot be excluded that the carcinogenicity seen after inhalation exposure would also occur following these other exposure routes.

The Dossier Submitter further assessed the carcinogenic potency of butanone oxime, apparently deriving an oral T25 value of 108.3 mg/kg bw/day based on the hepatocellular adenoma frequency seen in male rats. Such a value, according to the CLP guidance, may indicate low potency and support the setting of a ten-fold higher concentration limit than is set routinely for a category 1B carcinogen.

The T25 estimate of potency is the daily dose (in mg/kg bw) inducing a tumour incidence of 25 % over a lifetime exposure, and is based upon the assumption of a linear dose-response at all concentrations excluding the zero dose. As the Dossier Submitter did not provide the calculation they made to derive a T25 value of 108.3 mg/kg bw/day, RAC made an independent assessment.

The lowest exposure level causing a significant increase in liver tumours in the available studies was 270 mg/m^3, at which 9.8 % of male rats developed liver cell adenoma. As there were no such tumours in control rats, this was the net tumour incidence at this exposure level in this study. Exposures were made 6 h/day, 5 days/week for 26 months (i.e. slightly longer than the standard 24 month lifetime period).

The T25 (inhalation, rat) from this study for a period of 6 h/day, 5 days/week (26 months) would therefore be approximately 690 mg/m^3, or 492.9 mg/m^3 for exposure 6 h/day, 7 days/week (26 months). This is in agreement with the assessment made by the Dossier Submitter.

Assuming that adult rats breathe 0.006 m^3 of air per hour, when they are exposed for 6h/day, they are receiving 36 L/day ≡ 0.036 m^3/day. Therefore, assuming body weights of 0.5 kg, and 100 % absorption of butanone oxime by the oral route compared to 100 % following inhalation exposure:

T25 (oral, rat) = 492.9 × 0.036/0.5 × 100/100
approximately = 35.4mg/kg bw/day

This is a considerably lower value than the T25 of 108.3 mg/kg bw/day derived by the Dossier Submitter. As a T25 of 35.5 lies between 1 and 100 mg/kg bw/day, butanone oxime should be regarded as a medium potency carcinogen; the setting of a specific concentration limit would be inappropriate. There are no additional factors relevant for butanone oxime to justify a further extrapolation, although the 26-month study duration implies a slightly lower potency for a standard 24 month period than calculated. Strictly, the value calculated by the Dossier Submitter could indicate that butanone oxime be regarded as a low potency carcinogen and, if supported, this could support the setting of a higher specific concentration limit (e.g. 1%). However, this value is close to the upper boundary for a medium potency carcinogen and, as the Dossier Submitter did not provide or justify their calculation method, it does not provide a robust basis to increase the concentration limit.

RAC therefore concludes that **classification of butanone oxime in category 1B; H350 (May cause cancer)** for carcinogenicity would be appropriate, and that the general concentration limit of 0.1 % should apply.

4.11 Toxicity for reproduction

Table 29: Summary table of relevant reproductive toxicity studies (fertility)

Method	Results	Remarks	Reference

Two-generation toxicity study **Oral (gavage),** EPA guideline with modifications (similar to OECD TG 416/EU B.35), GLP compliant **Rat**; CD Sprague-Dawley (Crl:CD[SD]BR) VAF/Plus); male/female (30/sex/dose) Doses tested: 0, 10, 100, 200 mg/kg bw/day (actual ingested) Exposure regimen: F0 generation: starting from 8 wk of age during 10 wk pre-mating (5d/wk); 3 wk mating period to produce the F1 generation, gestation, and lactation for 7 d/wk (continued dosing)	**Parental parameters** **<u>200 mg/kg bw/d:</u>** <u>*Mortality:*</u> 4/30 (13.3 %) F0m, including 3 during pre-breed period; 11/30 (36.7 %) F0f; 15/30 (50.0 %) F1m including 8 during pre-breed period, 1 during the first bred, 3 during the second breed; 8/30 (26.7 %) F1f including 4 during the pre-breed period, 3 during the vaginal cytology (first breed) and 1 during the lactation of her F2b litter <u>*Clinical signs:*</u> ↓ bw and bw gain in both generations and m/f; ↓ feed consumption in F0m/f + F1f; F0m: tremors, salivation, slow respiration, mouth breathing, lethargy, staggers, and rooting in bedding (post-dosing, presumably from the "taste" of the dosing solution); F0f: tremors, ataxia, and convulsions (only in animals prior to demise), stupor, abnormal respiration (audible, irregular, raspy, laboured), dyspnoea, dehydration, excessive urination, bright yellow urine, and rooting in bedding; F1m: tremors, audible breathing, and rooting in bedding; F1f: lethargy, abnormal respiration (laboured, gasping, and raspy), cyanosis, and rooting in bedding <u>*Haematology:*</u> F0m/f + F1m/f: anaemia with ↓: RBC count, Hct, Hb, ↑: RBC size (MCV), nucleated RBC count, reticulocyte count, MCH, WBC count; F0m + F1m: methaemoglobin <u>*Necropsy:*</u> F0m/f + F1m/f: ↑ abs. and rel. (significant) spleen weight; F1m + F0f + F1f: ↑ rel. (significant) liver weight <u>*Histology:*</u> F0m/f + F1m/f: spleen: congestion, extramedullary haematopoiesis and haemosiderosis, liver: extramedullary haematopoiesis and haemosiderosis **<u>100 mg/kg bw/d:</u>** <u>*Clinical signs:*</u> F0m: lethargy, staggers, and rooting in bedding; F0f: weaving, tremors, and rooting in bedding; F1m: slight dehydration, audible breathing, rooting in bedding; F1f: laboured breathing <u>*Haematology:*</u> F0m/f + F1m/f: anaemia with ↓: RBC count, Hct, Hb, ↑: RBC size (MCV), nucleated RBC count, reticulocyte count, MCH; F0m + F1m: ↑: WBC count, methaemoglobin <u>*Necropsy:*</u> F0m/f and F1m/f: ↑ abs. and rel. (significant) spleen weight; <u>*Histology:*</u> F0m/f and F1m/f: spleen and liver: extramedullary haematopoiesis and haemosiderosis **<u>10 mg/kg bw/d:</u>** <u>*Clinical sign:*</u> F0m + F1m: rooting in bedding <u>*Haematology:*</u> F0m: ↓ RBC count, Hb <u>*Necropsy:*</u> F0m: dark spleens (5/30) <u>*Histology:*</u> F0 m/f + F1m/f: spleen and liver: extramedullary haematopoiesis and haemosiderosis **$LOAEL_{sys\ m/f}$ = 10 mg/kg bw/d (toxicity to the haematopoietic system)** based on effects in the spleen: hematopoietic cell proliferation, pigmentation and congestion and liver: haematopoiesis and pigmentation in both sexes of F0 and F1 adults	**Key study** Test material: butanone oxime, purity: > 99 %	TL17 (1992), unpublished study report, confidential; Tyl et al. (1996)

Method	Results	Remarks	Reference
	Reproductive parameters - no significant effects of treatment on number of F0 or Fl females, pre- or post-breed, which were cycling, or on cycle length, number of females not cycling, or number of females with abnormal cycles - no effects at any dose for F0 and Fl (a + b) generations for any reproductive indices - prenatal mortality and stillbirth indices exhibited no significant trends or pair wise comparisons, although there appeared to be slight dose-related increases for both parameters in F0 matings and for stillbirth index only in Fl (a + b) matings, but were well within the historical control data **Offspring parameter** - no effects of treatment at any dose on total or live litter size, sex ratio, or pup body weights per litter, with sexes pooled or separate (pnd 0—21) for Fl and F2 (a + b) litters **NOAEL = 200 mg/kg bw/d for reproductive toxicity (fertility)** According to CLP: **no classification for reproductive toxicity**		

Table 30: Summary table of relevant developmental toxicity studies

Method	Results	Remarks	Reference
Two-generation toxicity study Oral (gavage), EPA guideline with modifications (similar to OECD TG 416/EU B.35), GLP compliant Rat; CD Sprague-Dawley (Crl:CD[SD]BR) VAF/Plus); male/female (30/sex/dose) Doses tested: 0, 10, 100, 200 mg/kg bw/day (actual ingested) Exposure regimen: F0 generation: starting from 8 wk of age during 10 wk pre-mating (5d/wk);	Parental parameters 200 mg/kg bw/d: *Mortality:* 4/30 (13.3 %) F0m, including 3 during pre-breed period; 11/30 (36.7 %) F0f; 15/30 (50.0 %) F1m including 8 during pre-breed period, 1 during the first bred, 3 during the second breed; 8/30 (26.7 %) F1f including 4 during the pre-breed period, 3 during the vaginal cytology (first breed) and 1 during the lactation of her F2b litter *Clinical signs:* ↓ bw and bw gain in both generations and m/f; ↓ feed consumption in F0m/f + F1f; F0m: tremors, salivation, slow respiration, mouth breathing, lethargy, staggers, and rooting in bedding (post-dosing, presumably from the "taste" of the dosing solution); F0f: tremors, ataxia, and convulsions (only in animals prior to demise), stupor, abnormal respiration (audible, irregular, raspy, laboured), dyspnoea, dehydration, excessive urination, bright yellow urine, and rooting in bedding; F1m: tremors, audible breathing, and rooting in bedding; F1f: lethargy, abnormal respiration (laboured, gasping, and raspy), cyanosis, and rooting in bedding *Haematology:* F0m/f + F1m/f: anaemia with ↓: RBC count, Hct, Hb, ↑: RBC size (MCV), nucleated RBC count, reticulocyte count, MCH, WBC count; F0m + F1m: methaemoglobin *Necropsy:* F0m/f + F1m/f: ↑ abs. and rel. (significant) spleen weight; F1m + F0f + F1f: ↑ rel. (significant) liver weight *Histology:* F0m/f + F1m/f: spleen: congestion, extramedullary haematopoiesis and haemosiderosis, liver: extramedullary haematopoiesis and haemosiderosis 100 mg/kg bw/d: *Clinical signs:* F0m: lethargy, staggers, and rooting in bedding; F0f: weaving, tremors, and rooting in bedding; F1m: slight	Test material: butanone oxime, purity: > 99 %	TL17 (1992), unpublished study report, confidential; Tyl et al. (1996)

Method	Results	Remarks	Reference
3 wk mating period to produce the F1 generation, gestation, and lactation for 7 d/wk (continued dosing); F0 and F1 weanlings were necropsied after 2 wk post wean F1 generation: starting from 11 wk of age in the same regime (5 d/wk);	dehydration, audible breathing, rooting in bedding; F1f: laboured breathing *Haematology:* F0m/f + F1m/f: anaemia with ↓: RBC count, Hct, Hb, ↑: RBC size (MCV), nucleated RBC count, reticulocyte count, MCH; F0m + F1m: ↑: WBC count, methaemoglobin *Necropsy:* F0m/f and F1m/f: ↑ abs. and rel. (significant) spleen weight; *Histology:* F0m/f and F1m/f: spleen and liver: extramedullary haematopoiesis and haemosiderosis 10 mg/kg bw/d: *Clinical sign:* F0m + F1m: rooting in bedding *Haematology:* F0m: ↓ RBC count, Hb *Necropsy:* F0m: dark spleens (5/30) *Histology:* F0 m/f + F1m/f: spleen and liver: extramedullary haematopoiesis and haemosiderosis $LOAEL_{sys\ m/f}$ = 10 mg/kg bw/d (toxicity to the haematopoietic system) based on effects in the spleen: hematopoietic cell proliferation, pigmentation and congestion and liver: haematopoiesis and pigmentation in both sexes of F0 and F1 adults Reproductive parameters - no significant effects of treatment on number of F0 or Fl females, pre- or post-breed, which were cycling, or on cycle length, number of females not cycling, or number of females with abnormal cycles - no effects at any dose for F0 and Fl (a + b) generations for any reproductive indices - prenatal mortality and stillbirth indices exhibited no significant trends or pair wise comparisons, although there appeared to be slight dose-related increases for both parameters in F0 matings and for stillbirth index only in Fl (a + b) matings, but were well within the historical control data Offspring parameter - no effects of treatment at any dose on total or live litter size, sex ratio, or pup body weights per litter, with sexes pooled or separate (pnd 0—21) for Fl and F2 (a + b) litters - no treatment-related clinical observations for Fl or F2 (a + b) pups during lactation - no treatment-related necropsy findings of pups during lactation or of Fl or F2a pups, 10/sex/dose, which were necropsied at weaning - no treatment-related changes in haematology or organ weights in Fl, F2a, or F2b weanlings, 10/sex/dose Overall, no evidence of postnatal toxicity was found at any dose tested.		
Developmental toxicity study, according to OECD TG 414/ EU B.31, GLP compliant Oral (gavage) Rat; Sprague-Dawley , female (25/dose) Doses tested: 0, 60, 200, 600 mg/kg bw/day; dose volume: 10 mL Exposure regimen:	Preliminary study (dose range-finding study) NOAEL = 400 mg/kg bw/d for developmental toxicity, based on all gestational parameters evaluated during caesarean section including viable foetuses, early and late resorptions, foetal sex ratios, gravid uterus weights and foetal body weights; no foetal external malformations or developmental variations $LOAEL_{sys\ f}$ = 25 mg/kg bw/d for maternal toxicity (toxicity to the haematopoietic system) based on signs of anaemia (↑ methaemoglobin (GD16/20: 6/4 %), ↑ reticulocyte (GD16/20: 18/14 %) Main study NOAEL = 600 mg/kg bw/d for developmental toxicity, based on any parameters evaluated during caesarean section including the number of corpora lutea, implantation sites, viable foetuses, resorptions, foetal sex ratios, and foetal body weights; no treatment-related foetal malformations; no visceral or skeletal malformations $LOAEL_{sys\ f}$ = 60 mg/kg bw/d for maternal toxicity (toxicity to the haematopoietic system) based on spleen enlargement According to CLP: no classification for developmental toxicity	Key study Test material: butanone oxime, purity: > 99 %	TL19 (1990a), unpublished study report, confidential; Derelanko et al. (2003)

Method	Results	Remarks	Reference
GD6-15 (daily), observation period: until sacrifice on GD20			
Developmental toxicity study, according to OECD TG 414/ EU B.31, GLP compliant Oral (gavage) Rabbit; New Zealand White; female (18/dose) Doses tested: 0, 8, 14, 24, 40 mg/kg bw/day; dose volume: 2 mL Exposure regimen: GDs 6-18 (daily), observation period: until sacrifice on GD29	<u>Preliminary study (dose range-finding study)</u> 80 mg/kg bw/d: mortality in 5/5 dams between GD8-10; first mortality: ≤ 48h in 2/5 females (see Section '4.2 Acute toxicity') 40 mg/kg bw/d: mortality in 2/5 on GD10 or 11, 1 dam aborted on GD20, 2/5 survived to scheduled sacrifice on GD29 20 and 40 mg/kg bw/d: 100 % pregnancy rate 10 and 80 mg/kg bw/d: 60 % pregnancy rate Control: 80 % pregnancy rate <u>Main study</u> 40 mg/kg bw/d on GD6-18: mortality in 8/18 dams (44 %) between GD11-24; 3 abortions LOAEL = 40 mg/kg bw/d for developmental toxicity, based on abortions in 3/10 adult females in pregnant rabbits NOAEL = 24 mg/kg bw/d for developmental toxicity, based on any treatment-related gestational effects, malformations or developmental variations (↓ mean number of viable foetuses of 5.3, but fell in the historic control range of 4.6-9.1); not noted at 20 mg/kg bw/d in the preliminary study $LOAEL_{sys\ f}$ = 10 mg/kg bw/d for maternal toxicity (toxicity to the haematopoietic system) based on signs of anaemia in the dams (increase in methaemoglobin and reticulocytes appeared at GD13 and progressively increased with time until GD19 (3 days after the end of exposure)) According to CLP: no classification for developmental toxicity	Key study Test material: butanone oxime, purity: > 99 %	TL19 (1990b), unpublished study report, confidential; Derelanko et al. (2003)

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Butanone oxime was tested for effects on sexual function and fertility in a two-generation toxicity study in rats (similar to OECD TG 416/EU B.35). The study was performed to evaluate the potential of butanone oxime administered by gavage to CD Sprague-Dawley rats to produce alterations in parental fertility, maternal pregnancy and lactation, and growth and development of the offspring for two generations, one litter per generation for the F0 to F1 generation, and at least one litter per generation in two breedings for the F1 to F2 generation. In the study butanone oxime was used as test material. The results of the experimental study on fertility are summarised in Table 29 (see there).

4.11.1.2 Human information

No information is available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Butanone oxime was tested for adverse effects on development in developmental toxicity studies (similar to OECD TG 414/EU B.31) in rats and rabbits. In both species a preliminary dose range-finding study and a main study were performed. Sprague-Dawley rats were administered butanone oxime oral by gavage during GD6–15 and New Zealand White rabbits were treated during GD 6-18. In both studies butanone oxime (purity: confidential) was used as test material. The results of these experiments on developmental toxicity are summarised in Table 30 (see there).

4.11.2.2 Human information

No information is available.

4.11.3 Other relevant information

No information is available.

4.11.4 Summary and discussion of reproductive toxicity

Effects on sexual function and fertility: Data on reproductive toxicity was obtained from animal testing. No information is available on effects of butanone oxime on sexual function and fertility in humans.

In a two-generation toxicity study, with one breed for the first generation and two breeds (the second for initially unsuccessful animals) for the second generation the effects of butanone oxime on sexual function and fertility was examined in rats. Toxicity in adult animals was noted in both generations and both sexes. Treatment-related parental deaths occurred at 200 mg/kg bw/d. At 100 and 200 mg/kg bw/d signs of haemolytic anaemia and compensatory erythropoiesis was present and contributed to the increased spleen weights and extramedullary haematopoiesis (hematopoietic cell proliferation) and haemosiderosis (pigment deposition from haemoglobin breakdown products) in spleens and livers. At 10 mg/kg bw/d the consistent parental findings were extramedullary haematopoiesis and haemosiderosis in spleens and livers unaccompanied with further lesions. A NOAEL for systemic effects of adult toxicity could not be established in this study. There were no treatment-related effects on parental reproductive parameters, on parental reproductive behaviour or on parental reproductive organ histology in rats dosed by gavage up to 200 mg/kg bw/d butanone oxime. There were no treatment-related effects on any offspring parameters, including pre- and postnatal survival and growth, for either generation. For butanone oxime a NOAEL of 200 mg/kg bw/d for reproductive toxicity in rats was established.

Effects on development: Data on developmental toxicity was obtained from animal testing. No information is available on effects of butanone oxime on development in humans.

Effects of butanone oxime on development were investigated in rats and rabbits. In Sprague-Dawley rats and New Zealand White rabbits results of a preliminary dose range-finding study and of the main study are available. Based on the results of these studies, butanone oxime is not considered to be developmentally toxic at maternally toxic dose levels of up to 600 mg/kg bw/d in the rat. NOAEL values of 400 and 600 mg/kg bw/d for developmental toxicity, based on absence of treatment-related

gestational effects, malformations or developmental variations at the highest dose tested, could be derived in these studies. For maternal toxicity LOAEL values of 25 mg/kg bw/d (preliminary dose range-finding study), based on signs of anaemia, and 60 mg/kg bw/d (main study), based on spleen enlargement could be established.

Rabbits proved to be more sensitive to butanone oxime toxicity. Butanone oxime was significantly more toxic to the rabbit than the rat. Three rabbits aborted and 8/18 females, which have received oral doses of 40 mg/kg bw/d by gavage during the gestation phase, were found dead between GD11-24. The preliminary study in rabbits showed maternal toxicity indicative of haemolytic anaemia (increases in methaemoglobin and reticulocytes) at 10 mg/kg bw/d and higher. No treatment-related gestational effects, malformations or developmental variations were observed in the rabbit at dose levels at or below 24 mg/kg bw/d.

The results available from examination the effects on sexual function and fertility in rats and on developmental toxicity in rats and rabbits do not provide information concerning butanone oxime as a reproductive or developmental toxicant.

4.11.5 Comparison with criteria

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. In this classification system, reproductive toxicity is subdivided under two main headings: (a) Adverse effects on sexual function and fertility; (b) Adverse effects on development of the offspring.

Classification is made on the basis of the appropriate criteria and assessment of the total weight of evidence. Classification as reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction, e.g. adverse effects on sexual function and fertility, or on development of the offspring, and in addition adverse effects on or via lactation, and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

According to CLP the following applies for the classification of a substance as reproductive toxicants:

- *Hazard categories for reproductive toxicants*

'Category 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primary from human data (Category 1A) or from anima data (Category 1B).'

'Category 1A: Known human reproductive toxicant

The classification of a substance in Category 1A is largely based on evidence from human.

No data are available which justify a classification of butanone oxime as 'Category 1A, known human reproductive toxicant' in accordance with CLP.

'Category 1B: Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on evidence from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.'

The study results available from examination the effects on sexual function and fertility in rats and on developmental toxicity in rats and rabbits have not provide information concerning butanone oxime as a reproductive or developmental toxicant in humans.

'Category 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.'

Data on reproductive toxicity (effects on sexual function and fertility) and on developmental toxicity was obtained from animal testing.

<u>Effects on sexual function and fertility:</u>

In the available two-generation toxicity study in the rat no reproductive toxicity, e.g., effects on parental reproductive parameters, on parental reproductive behaviour or on parental reproductive organ histology, was observed at 200 mg/kg bw/d, the highest dose studied. Toxicity to the haematopoietic system was observed in adults at all doses studied (≥ 10 mg/kg bw/d).

In conclusion, available results of a study examining effects of butanone oxime on sexual function and fertility in rats give no indications that butanone oxime is a reproductive toxicant.

<u>Effects on development:</u>

In rats no developmental toxicity was noted at the highest dose tested of 600 mg/kg bw/d. Maternal toxicity indicative of haemolytic anaemia occurred at all dose tested (≥ 10 mg/kg bw/d).

In rabbits no developmental toxicity was observed in the absence of excessive maternal toxicity. At the highest dose tested of 40 mg/kg bw/d excessive mortality and abortions in 3/10 adult pregnant rabbits occurred in unreliable results. Maternal toxicity (toxicity on the haematopoietic system) occurred at all dose tested (≥ 10 mg/kg bw/d).

In conclusion, available results of studies examining effects of butanone oxime on developmental toxicity in rats and rabbits give no indications that butanone oxime is a developmental toxicant.

- *Hazard category for lactation effects*

'Effects on or via lactation:

Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.'

The data on effects on or via lactation was obtained from animal testing.

A two-generation toxicity study in the rat, which involves direct exposure or exposure via the milk of the offspring postnatally, provided information on effects on or via lactation. Direct observations of the pups during lactation have not shown adverse effects, such as deaths, decreased viability, clinical signs such as reduced bodyweight gain etc. An impaired nursing behaviour was not reported.

Data available from the two-generation study with butanone oxime in rats do not provide evidence of adverse effects on the offspring due to transfer in the milk.

4.11.6 Conclusions on classification and labelling

Sexual function and fertility:

Well-documented test results from a study on sexual function and fertility in adult male and female rats provide no evidence of butanone oxime-induced adverse effects. According to CLP, classification of butanone oxime as reproductive toxicant (fertility) is not warranted.

Developmental toxicity:

Well-documented test results from studies on development of the offspring from rats and rabbits provide no evidence of butanone oxime-induced adverse effects.

According to CLP, classification of butanone oxime as developmental toxicant is not warranted.

Lactation:

Results from a two-generation study with butanone oxime in rats have not provided clear evidence of adverse effects on the offspring due to transfer in the milk.

According to CLP, classification of butanone oxime as reproductive toxicant via lactation is not warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Data on reproductive and developmental toxicity were obtained from studies in animals. No information was available on effects of butanone oxime on sexual function and fertility or on development in humans.

Sexual function and Fertility was examined during a two-generation toxicity study in rats. Toxicity was noted in both generations and in both sexes. Findings indicative of haemolytic anaemia were noted from doses of 10 mg/kg bw/day in parents and there was an increase in treatment-related parental deaths at 200 mg/kg bw/day. There were no treatment-related effects on any offspring parameters, including pre- and postnatal survival and growth for either generation. No classification for sexual function and fertility is proposed.

The effect of butanone oxime on development was investigated in studies with rats and rabbits. Despite the presence of maternal toxicity in both studies, no gestational effects, malformations or developmental variations were observed. No classification for developmental toxicity is proposed.

According to the DS, butanone oxime should not be classified for effects on sexual function and fertility, developmental toxicity or lactation.

Comments received during public consultation

One MS provided a comment of support for no classification for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

Sexual Function and Fertility

In a two-generation toxicity study, male and female Sprague-Dawley rats (30/sex/dose) were administered butanone oxime at doses of 0, 10, 100 or 200 mg/kg bw/day by gavage. F0 animals were dosed for a period of 10 weeks prior to mating (5 days/week), for 3 weeks during the mating period to produce the F1 generation, during gestation, and during lactation (7/days per week). F1 parents underwent a first breeding to produce the F2a generation and for animals unsuccessful in the first breeding, a second was carried out to produce the F2b generation (no further details on this were provided).

Significant parental toxicity was observed in males and females of the top two doses. At 200 mg/kg bw/day there was an increase in mortality occurring in the pre-breeding period up until the end of the study. Body weight and body weight gain was reduced in males and females of both generations (details not specified) and animals showed clinical signs such as tremors, salivation, slow respiration and staggering. There were indications of haemolytic anaemia in males and females of all dose groups.

There were no effects on reproductive parameters in this study. No effects on cycling or cycle length and no effect on reproductive indices such as fertility index or lactation index were noted. There was a slight dose-related increase in prenatal mortality and still-birth indices during F0 matings, however these were described as being well within historical control data limits. In the F1 (a and b) matings there was a non-statistically significant increase in still-birth index but this was again described as being well within the HCD. No further details were provided on these endpoints.

There were no effects on offspring parameters such as total or live litter size, sex ratio or pup weights per litter.

The results of this study indicate there were no treatment-related effects on sexual function or fertility. There is no evidence to support classification.

Developmental Toxicity

Two developmental toxicity studies are available, one in rats and one in rabbits.

In a developmental toxicity study in Sprague Dawley rats, (6/dose in a preliminary study and 25/dose in the main study) pregnant animals were given doses of 0, 25, 100, 200 or 400 mg/kg bw/day (preliminary study) and 0, 60, 200 or 600 mg/kg bw/day (main study) butanone oxime by gavage. The exposure duration was GD 6-15.

Despite the presence of maternal toxicity at all doses tested (indicative of haemolytic anaemia) there were no signs of developmental toxicity in this study. All gestational parameters were similar to controls including the number of viable foetuses, early and late resorptions, foetal sex ratios and foetal body weights. There were no treatment-related malformations or variations in this study.

In the rabbit study, pregnant New Zealand White rabbits were dosed on GD 6-18 with butanone oxime in a preliminary study (doses: 0, 10, 20, 40 or 80 mg/kg bw/day, 5/dose group) and in a main study (doses: 0, 8, 14, 24 and 40 mg/kg bw/day, 18/dose group). Pregnant rabbits appeared to be more sensitive to the effects of butanone oxime than rats and increased mortality was observed in both the preliminary study and the main study from doses of 40 mg/kg bw/day.

In the preliminary study, all 5/5 rabbits dosed with 80 mg/kg bw/day died by GD 10. At 40 mg/kg bw day, 2/5 rabbits died by GD 11 and one dam aborted her entire litter on GD 20.

In the main study, 8/18 dams died between GD 11-24. Three of the remaining rabbits aborted their litters.

Signs of anaemia were present from doses of 10 mg/kg bw/day.

There were no findings relating to treatment on the incidence of malformations or variations in foetuses at doses of 24 mg/kg bw/day or below. Details above this dose are not available; however excessive mortality and abortions at this dose preclude any meaningful assessment of the caesarean section.

The data available from two developmental toxicity studies, one in rats and one in rabbits, do not provide evidence for classification for developmental toxicity.

RAC is in agreement with the Dossier Submitter that **no classification for toxicity to sexual function and fertility or development** is required.

4.12 Other effects

Not evaluated in the scope of this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in the scope of this dossier.

6 OTHER INFORMATION

None

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Registration dossier Butanone Oxime: https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/1

8 ANNEXES

Confidential Annex

ABBREVIATIONS and ACRONYMS

abs.	Absolute
ATE	Acute Toxicity Estimates value; Dose/concentration that cause mortality
bw	Body weight
C	Carbon
C&L	Classification and labelling
CAS	Chemical abstracts service
CLH	Harmonised classification and labelling
CLP	Regulation (EC) No 1272/2008 of the European Parliament and of the Council of December 2008 on classification, labelling and packaging of substances and mixtures
CNS	Central nervous system

Dgr	Danger
DNA	Deoxyribonucleic acid
EC	European Community
ECHA	European Chemicals Agency
e.d.	Epidermal
e.g.	for example
et al.	and others
f/F	Female
GD	Gestation day
GHS05	Hazard pictogram, Symbol: Corrosion
GHS06	Hazard pictogram, Symbol: Skull and crossbones
GHS07	Hazard pictogram, Symbol: Exclamation mark
GHS08	Hazard pictogram, Symbol: Health hazard
GPMT	Guinea Pig Maximisation Test
h	Hour
H	Hazard statement
Hb	Haemoglobin
Hct	Haematocrit
i.d.	Intradermal
i.e.	id est (that is to say)
IUCLID	International uniform chemical information database
IUPAC	International union of pure and applied chemistry
kg	Kilogram
LC_{50}	50 % letal concentration, death of 50 % (one half) of a group of test animals
LD_{50}	50 % letal dose, death of 50 % (one half) of a group of test animals
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
m/M	Male
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume erythrocytes
MEK	Methyl ethyl ketone
MEST	Mouse Ear Swelling Test
mg/kg bw	Milligrams per kilogram body weight
mg/kg bw/d	Milligrams per kilogram body weight per day
mg/m^3	Milligrams per cubic meter
N	Nitrogen
neg.	Negative
No.	Number
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
O	Oxygen
OECD	Organisation for Economic Co-operation and Development
OECD TG	OECD Test Guideline
OH	Hydroxyl group
pH	Hydrogen ion concentration
ppm	Parts per million
pos.	Positive

REACH	Regulation (EC) No. 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
RBC	Red blood cell count
rel.	Relative
RNA	Ribonucleic acid
SEV	Substance evaluation
sig.	Significant
STOT RE	Specific target organ toxicity – repeated exposure
STOT SE	Specific target organ toxicity – single exposure
TG	Test group
TL	Test lab
UDS	Unscheduled DANN Synthesis Assay
WBC	White blood cell
wk	week(s)
↑	Increase
↓	Decrease