Committee for Risk Assessment

RAC

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

Adopted

14 September 2018

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14 September 2018

CLH-O-0000001412-86-227/F

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

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

Chemical name: **butanone oxime; ethyl methyl ketoxime; ethyl methyl ketone oxime**

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

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

The proposal was submitted by **Germany** and received by RAC on **18 May 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **19 July 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 September 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-014-00-0	butanone oxime; ethyl methyl ketoxime; ethyl methyl ketone oxime	202-496-6	96-29-7	Acute Tox. 4* Eye Dam. 1 Skin Sens. 1 Carc. 2	H312 H318 H317 H351	GHS08 GHS05 GHS07 Dgr	H312 H318 H317 H351			
Dossier submitters proposal	616-014-00-0	butanone oxime; ethyl methyl ketoxime; ethyl methyl ketone oxime	202-496-6	96-29-7	**Retain** Eye Dam. 1 **Add** Acute Tox. 3 STOT SE 3 **Modify** Acute Tox. 4 Skin Sens. 1B Carc. 1B	**Retain** H318 **Add** H301 H336 **Modify** H312 H317 H350	GHS06 GHS05 GHS08 Dgr	**Retain** H318 **Add** H301 H336 **Modify** H312 H317 H350		**Add** ATE oral, 100 mg/kg ATE dermal, 1 848 mg/kg	
RAC opinion	616-014-00-0	butanone oxime; ethyl methyl ketoxime; ethyl methyl ketone oxime	202-496-6	96-29-7	**Retain** Eye Dam. 1 Skin Sens. 1 **Add** Acute Tox. 3 Skin Irrit. 2 STOT SE 3 **Modify** Acute Tox. 4 STOT SE 1 STOT RE 2 Carc. 1B	**Retain** H318 H317 **Add** H301 H315 H336 H312 H370 (upper respiratory tract) H373 (blood system) H350	GHS06 GHS05 GHS08 Dgr	**Retain** H318 **Add** H317 H301 H315 H336 **Modify** H312 H350 H370 (upper respiratory tract) H373(blood system)		**Add** ATE oral, 100 mg/kg ATE dermal, 1 100 mg/kg	
Resulting Annex VI entry if agreed by COM	616-014-00-0	butanone oxime; ethyl methyl ketoxime; ethyl methyl ketone oxime	202-496-6	96-29-7	Carc. 1B Acute Tox. 3 Acute Tox. 4 STOT SE 3 STOT SE 1 STOT RE 2 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1	H350 H301 H312 H336 H370 (upper respiratory tract) H373(blood system) H315 H318 H317	GHS05 GHS06 GHS08 Dgr	H350 H301 H312 H336 H370 (upper respiratory tract) H373 (blood system) H315 H318 H317		ATE oral, 100 mg/kg ATE dermal, 1 100 mg/kg	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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 MSCA 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 MSCAs 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 MSCAs 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 MSCAs agreed with the proposed classification for acute dermal toxicity.

Three comments (2 MSCAs 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.

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)** with an **ATE of 100 mg/kg bw d**;
- **Acute Tox. 4; H312 (Harmful in contact with skin)** is appropriate with an **ATE of 1100 mg/kg bw**.

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 MSCA 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 MSCA 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).

Respiratory irritation

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.

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 MSCA was received in support of no classification for skin irritation, based on the results of the 2 studies described by the DS.

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.

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)**.

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 MSCAs, all in agreement that butanone oxime is a skin sensitiser. However, although one MSCA was in agreement that sub-categorisation with 1B was justified, the other three MSCAs 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.

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 MSCA 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) Equiv. OECD 408 GLP	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 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> <u>≥ 60 mg/kg bw/day:</u> No significant toxicity at this dose.	*≤ 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 % <u>Main study:</u> <u>≥ 200 mg/kg bw/day:</u> 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* <u>Preliminary study:</u> <u>10 mg/kg bw/day:</u> No significant toxicity at this dose. <u>40 mg/kg bw/day:</u> Mortality: 2/5 (GD 10 -11) Blood values: ↑ Reticulocytes ↑ Methaemo-globin (both reversible) <u>Main study:</u> <u>40 mg/kg bw/day:</u> Mortality:8/18 (GD 11-24) Red/green urine, pale eyes and ears, pale liver, brown dicoloured lungs.	*≤ 600 mg/kg bw/day* <u>Preliminary study:</u> <u>80 mg/kg bw/day:</u> 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 on 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.

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 MSCA 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**.

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 MSCAs 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 MSCA 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 6 h/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.

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.

Additional references

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

ANNEXES:

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

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

Annex 3 Records of the targeted public consultation (excluding confidential information)